Identification and Characterization of Quantitative Trait Loci (QTLs) Associated with Waterlogging Tolerance in Barley (*Hordeum vulgare* L.)

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DECLARATION

The thesis contains no material, which has been accepted for a degree or diploma by the University or any other institution, and to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due acknowledgement or reference is made in the text of this thesis.

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Hobart, June 2007

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List of Abbreviations

ADH	alcohol dehydrogenase
AE	additive × environment interaction effect
AFLP	amplified fragment length polymorphism
ANOVA	analysis of variance
AOX	alternative oxidase
ASREML	a statistics package performing restricted maximum likelihood
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
AUCPC	area under chlorosis progress curve
BC_2	backcross generation 2
BLUP	best-linear-unbiased prediction
bp	base pairs
BYDV	barley yellow dwarf virus
cDNA	complementary deoxyribonucleic acid
CIM	composite interval mapping
cM	centiMorgans
CTAB	cetyltrimethylammonium bromide
DArT	diversity array technology
dATP	deoxyadenosinetriphosphate
dCTP	deoxycytosinetriphosphate
dGTP	deoxyguanosinetriphosphate
dTTP	deoxythymidinetriphosphate
DE	dominance × environment interaction effect
df	degree of freedom
DH	doubled haploid
E. coli	*Escherichia coli*
EDTA	ethylenediamine tetra-acetic acid
EST	expressed sequence tag

FC	field capacity
Fm	maximum fluorescence
Fv	variable fluorescence
GA_3	gibberellic acid
GCA	general combining ability
GLM	general linear models
GSLs	general superior lines
h^2_B	broad-sense heritability
h^2_N	narrow-sense heritability
IE	epistasis × environment interaction effect
IM	interval mapping
kb	kilo base
LDH	lactate dehydrogenase
LOD	logarithm of odds
LR	likelihood ratio
LSD	least significant difference
MAS	marker-assisted selection
MCIM	mixed-model-based composite interval mapping
MQM	multiple QTL model
MS	mean square
PEPC	phosphoenolpyruvate carboxylase
PCR	polymerase chain reaction
PMT	photomultiplier
PPi	pyrophosphate
PROC CORR	a SAS procedure computes correlation co-efficient and simple descriptive statistics
PS II	photosystem II
QE	QTL × environment interaction effect
QTL	quantitative trait locus
RAPD	randomly amplified polymorphic DNA
REC	recombination estimates calculation

RFLP	restriction fragment length polymorphism
RILs	recombinant inbred lines
RuBPC	ribulose bisphosphate carboxylase
SAS	statistical analysis system
SCA	specific combining ability
SCAR	sequence characterised amplified region
SDRs	segregation distortion regions
SDS	sodium dodecyl sulfate
SE	standard error of a mean
SED	standard error of the difference between two means
SLs	superior lines
SNP	single nucleotide polymorphism
SOD	superoxide dismutase
SS	sum of squares
SSR	simple sequence repeat
STS	sequence tagged site
SuSy	sucrose synthase
TAE	tris-acetate-EDTA
TBE	tris-borate-EDTA
UDP	uridine diphosphate
Vr	variance
Wr	co-variance

Abstract

Waterlogging is a major environmental constraint severely limiting crop production both in Australia and worldwide. In Australia, most barley cultivars are waterlogging sensitive and increasing their tolerance is an important breeding objective in regions of high rainfall. However, little genetic research and progress has been made on improving barley for waterlogging tolerance, mainly because it is a complex abiotic stress that is affected by many factors such as temperature, plant development stage, nutrient, soil type and topography. The aims of this PhD project were to: (i) investigate the genetic behavior and quantitative inheritance of waterlogging tolerance in barley; (ii) identify and validate quantitative trait loci (QTL) for waterlogging tolerance in barley.

A quantitative genetic analysis of waterlogging was conducted with a 6 X 6 half diallel experiment between three Chinese tolerant cultivars and three susceptible Australian and Japanese cultivars. The six parents and 15 F_2 from each cross were seeded into two steel tanks (replications), flooded for 10 days and measured for mean yellow leaf percentage. This trait was chosen as other studies have found it to be correlated with waterlogging tolerance. The mean leaf chlorosis of all the F_2s was similar to that of their mid-parent values. Leaf chlorosis percentages followed an additive model with no significant dominance effect and possessed a high heritability. This experiment demonstrated that selecting in early generations for this trait would be effective.

For the purpose of identification of quantitative trait loci controlling waterlogging tolerance in barley, two linkage maps were constructed, based on doubled haploid populations from crosses between TX9425 (waterlogging tolerant) x Franklin (sensitive), and Yerong (tolerant) x Franklin. The TX9425/Franklin linkage map comprised 412 Diversity Array (DArT) markers, 27 SSR and 81 AFLP markers organized into 8 linkage groups and covering 956 cM. The Yerong/Franklin linkage map was based on 496 DArT and 22 SSR markers assigned to 9 linkage groups and covered 1084.5 cM. The robustness of the DArT markers was confirmed when linkage maps were generated from two sub-sets of progenies in the Yerong/Franklin population, which were genotyped in

different batches (arrays). The results indicated that the 496 markers were assigned to exactly the same seven different chromosomes in each of the two experiments. Only minor changes in marker order within chromosomes were found. A relatively large proportion of the molecular markers showed distorted segregation and the possible causes of this are discussed. In order to synthesize the genetic information contained in the two linkage maps produced in this project together with two other maps using DArT markers, a consensus map was constructed which could serve for barley molecular breeding in the future, and as a basis for studies of genome organization and evolution. For example, many more markers showed segregation distortion than expected. Out of the 2975 markers used across all four populations 21.1%, 10.9%, and 7.9% exhibited segregation distortion at 5%, 1%, and 0.5% probability threshold respectively. DArT markers were not more likely to show segregation distortion than other marker types. Of the 635 markers showing aberrant segregation in the four populations, 459 markers were located in 16 putative segregation distortion regions (SDR). The SDRs were identified on all seven barley chromosomes, but they were unevenly distributed over the seven chromosomes and their size varied from 4 to 46 cM. Ten of the SDRs were found in at least two populations and several at a consistent map location over the four populations. Further studies are needed to determine the molecular basis of segregation distortion.

In order to detect QTL for waterlogging tolerance in barley, the two mapping populations were tested for germination (six replicates of fifty seeds each, submerged in 50 ml of water for six days, then moved to incubators for germination), leaf chlorosis, biomass reduction and survival (four replicates (tanks), five plants from each DH and parental lines sown in pots, placed in tanks, three of which were flooded and one used as a control). QTL analysis found a total of 6 distinct QTLs for the four traits in the Franklin/TX9425 population and 6 QTLs in the Franklin/Yerong population. QTL controlling leaf chlorosis were quite similar in their location between the two populations, the most important QTL found being in the same region of chromosome 3H in each population. One QTL controlling plant survival rate was located on chromosome 2H in both populations. A QTL for biomass reduction was identified in each population to chromosome 4H. However, it may not be the same QTL because they mapped to slightly different regions of that chromosome in each population. A QTL for seed germination

was located on chromosome 1H in the Franklin/TX9425 population. However, this QTL was different from the QTL controlling leaf chlorosis, which mapped to a different region of the same chromosome. In this study strong QTLs were identified, which could be cross-validated in different mapping population for traits correlated with waterlogging tolerance in barley. Thus there is good scope for using marker assisted selection in breeding for waterlogging tolerance in barley. However, these QTL will need to be further validated through field experiments and yield measurements under waterlogging conditions.

Chapter 1 Introduction

Waterlogging is a condition of the soil where excess water in the root zone inhibits gas exchange with the atmosphere, and is often measured as the degree or depth of water saturation of the soil profile. Waterlogging stress occurs worldwide, strongly influencing natural and agricultural areas. It has been estimated that wetlands occupy approximately 6% of the earth's land surface (Maltby 1991). Waterlogging is widespread in Australia's dry land cropping environments as well as in irrigated areas in the southern regions of Australia (Moore and Mcfarlane 1998). Much of the Australian crop is grown on duplex soils, which have a layer of sandy soil over a relatively impermeable clay base, so that rainfall events can lead to rising water table in the root zone (Turner 1992). It has been estimated that annual crop production losses in Australia are A$180 million for waterlogging stress (Price 1993).

Barley, one of the most important crop species in the world, is very sensitive to waterlogging stress and can experience significant yield losses. Waterlogging is estimated to reduce yields by 20-25% overall with a range of 0-53% for waterlogging at different stages of development (Belford 1995). It would therefore be important to improve waterlogging and flooding tolerance in barley.

Due to the variable environments in the field and its complex quantitative character, little progress has been made on improvement of barley varieties for waterlogging tolerance. Limited genetic studies of barley waterlogging tolerance have been carried out until now due to the lack of an efficient index for measuring waterlogging tolerance. An overwhelming proportion of barley waterlogging studies have concentrated on morphological and physiological response to this trait. The physiological mechanisms involved in the response to waterlogging stress are complex, with most of the studies about barley waterlogging tolerance being reviewed in the next chapter.

Various morphological, physiological and biochemical responses were reported when barley plants were subjected to waterlogging stress (Pang *et al.* 2004, 2005). Some of these responses such as the chlorophyll content, net CO_2 assimilation, measurement of net O_2 and ion fluxes from the root surface and investigation of formation of aerenchyma

in adventitious roots are difficult to measure in field conditions. It is also not practical for breeders to use these indices to measure thousands of lines in a restricted period of time.

A few reports have demonstrated the genetic variation of the tolerance to waterlogging in cereal crops. Most of the early published studies on waterlogging tolerance in barley and wheat measured waterlogging tolerance using leaf chlorosis or leaf/plant death (Hamachi et al.1989; Cao et al. 1992, 1994, 1995; Cai et al. 1996). These results indicated that screening for waterlogging tolerance by the amount of dead leaves was a useful criterion.

Genetic differences for waterlogging tolerance have also been demonstrated based on yield and yield componenets. The first work to evaluate waterlogging tolerance based on plant grain yield was done by Bao (1997) using 20 wheat varieties. Xue et al (1997) also reported that there is an obvious difference in waterlogging tolerance among 20 different barley cultivars (lines), the authors found that some cultivars including Weisubuzi, Su5078, Tong83-11, Tong88-58 have better tolerance to waterlogging stress than others based on grain yield and yield components (Xu et al. 1997). Yang et al. (1999) compared the waterlogging tolerance of eight barley dwarf-mutants. The results showed that physiological and biochemical characters such as green leaf number of main stem, fresh weight of plant, activities of superoxide dismutase (SOD) in flag leaf were greatly changed by waterlogging stress, which also resulted in a decrease in grain yield. Van Ginkel (1992) demonstrated that there is a high negative correlation between leaf chlorosis (or death) and grain yield in wheat. Setter et al. (1999) demonstrated a genetic diversity of waterlogging tolerance in barley exposed to intermittent waterlogging over 4 weeks, and waterlogging tolerance was assessed using leaf chlorosis following waterlogging. According to their results, grain yield of barley was reduced by 51-84% of non-waterlogged plants, but the yield reduction did not coincide with severity of leaf chlorosis. It is not surprising because many other factors may affect yield during the long recovering period after waterlogging stress was determined, so yields were just partially correlated with the immediate response index such as leaf chlorosis even under uniform conditions.

Some of the previous studies have indicated that waterlogging tolerance is under genetic control, and it is heritable, with the broad sense heritability estimated to be over 70% (Cao et al. 1992, 1994, 1995; Cai et al. 1996; Bao et al. 1997). These authors concluded

that it is possible to improve waterlogging tolerance in wheat by selecting progeny in early generations based on related traits. Cao et al. (1992, 1995) found that waterlogging tolerance based on leaf chlorosis was controlled by one dominant gene, but tolerance based on traits such as green leaves/main stem, plant height, grains per ear and 1000-grain weight could be controlled by multiple genes in the varieties involved in their study (Cao. et al. 1994). Boru (1996) proposed that these different genes could relate different mechanisms of tolerance to waterlogging, therefore waterlogging tolerance could be substantially improved by combining all tolerance genes into one genotype.

Waterlogging is a complex abiotic stress encoding a large number of mechanisms and complicated by many confounding factors such as temperature, plant development stage, nutrient, soil type and sub-topography. Accuracy of field-testing results can always be compromised because these cofactors fluctuate both spatially and temporarilly during the course of an experiment in the field. It is difficult for plant breeders to introduce this trait into otherwise elite genotypes due to the low efficiency of direct selection of waterlogging tolerant plants in the field.

The development of dense genetic linkage maps, based on various molecular marker systems such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP), and diversity array technology (DArT) has permitted the detection of quantitative trait loci (QTL) for a range of characters in cultivated barley and has made marker assisted selection (MAS) potentially of great value for improving some complex and economically important traits in practical breeding programs. Due to its non-dependence on sequencing information, high throughput, low cost, and easy conversion into other types of markers (Jaccoud et al. 2001; Wenzl et al. 2004), DArT could enhance the utility of marker assisted selection in barley breeding programs. This makes it possible to study genetic mechanisms of barley waterlogging tolerance, and use marker assisted selection as an efficient way to bring waterlogging tolerance into commercial barley varieties.

This project aimed to investigate the genetic mechanism of waterlogging tolerance in barley. The research undertaken in this project is presented in seven chapters. Chapter 1 contains a general introduction to this project. Chapter 2 contains a review of relevant

literature on waterlogging tolerance in cereal crops. Chapter 3 describes a study of the genetic behaviour of barley waterlogging tolerance. Chapter 4 covers the construction of genetic linkage maps of two barley doubled haploid (DH) populations. Chapter 5 concentrates on the development of a barley consensus map based on four barley crosses, and the comparison of segregation distortion regions (SDRs) among different populations. Chapter 6 focuses on the identification and verification of quantitative trait loci (QTLs) controlling waterlogging tolerance in barley. The thesis concludes with a general discussion in chapter 7. Key findings are summarized and conclusions from the study are drawn, with further research directions suggested based on the project findings.

Chapter 2 Literature Review

Waterlogging occurs worldwide, strongly influencing natural and agricultural areas. It is widespread in Australia's dryland cropping environments as well as in irrigated areas in the southern regions of Australia (Moore and Mcfarlane 1998). Much of the Australian crop is grown on duplex soils, which have a layer of sandy soil over a relatively impermeable clay base, so that rainfall events can lead to rising water table in the root zone (Turner 1992). It has been estimated that annual crop production losses in Australia are A$180 million for waterlogging stress (Price 1993). Waterlogging also causes problems in regions with heavy textured soils in North America and Central Europe. Boyer (1982) estimated that waterlogging affected around 16% of the soils in the USA. It is now increasingly becoming a matter of major concern in many agricultural areas (Ghassemi et al. 1995), such as in irrigated areas of India, Pakistan and China (Crosson and Anderson 1992).

This thesis reports on part of a larger project to examine genetic and physiological mechanisms of waterlogging tolerance in barley, and to apply this knowledge to improved outcomes for plant breeders. A parallel part of the project examines physiological mechanisms of tolerance (Pang et al. 2004; Pang et al. 2006). The experimental studies reported in this thesis concentrate on understanding genetic control, and examine the use of molecular markers to assist incorporation of waterlogging tolerance into barley breeding programs. The following sections review aspects of agronomy, anatomy and physiology of waterlogging tolerance to assist later examination of its genetic control in barley.

2.1 Effects of waterlogging on soil properties

Soil aeration is closely connected with air-water relationships in soils. These affect biological activity of soil organisms, mainly microorganisms which are very sensitive to oxidation or reduction processes (Gliński and Stępniewski 1985). Redox status is a base for understanding soil properties, such as composition of soil solution, soil reaction, availability of water, gaseous emission to the atmosphere, stability of metal organic compounds, electrokinetic properties, surface charge, biological activity, etc. (Carter

1980; Engler and Patrick 1974; Gliński and Stępniewski 1985; Hesse 1971; Jeffrey 1960; Kauncher *et al.* 1974; Patrick and Jugusjinda 1992; Ponnamperuma 1986; Van Cleemput *et al.*1976; Yu 1985; Gliński *et al.* 2000). The reduced forms of elements are often toxic for plants and other living organisms, can pollute the environment and will increase greenhouse gas concentration in the atmosphere. The status of soil redox processes is expressed by the redox potential (Eh). The ability of the soil to maintain its redox potential is a measure of soil resistance to reduction (Gliński and Stępniewski 1986; Stępniewska 1988). Soil can counteract changes in its redox potential (redox buffering). Soil redox buffering capacity is a result of microbial activity, carbon availability, temperature, and of the oxidizing forms of nitrogen, manganese, iron, sulphur and phosphorus (Gliński et al. 2004).

2.2 Effects of waterlogging on barley plants

2.2.1 Response of barley roots to waterlogging stress

Because the root is the plant organ which is directly exposed to oxygen deficiency and soil reduction caused by waterlogging, the growth and activity of root systems are likely to be the first indicators of plant stress. Root growth reduction resulting from short-term flooding of barley was found to occur at all stages of plant development. When waterlogging commences in the very early growth stages of the plant, and is of sufficient intensity and duration, root development is reduced by retarded growth and death of part or all of the root system (Kramer 1951; van't Woudt and Hagan 1957; Crawford 1967; Smith and Robertson 1971). According to the results of Watson et al. (1976), after two weeks of continuous waterlogging, adventitious roots emerged from the stems above the water line. Some of the roots floated in the water and did not penetrate the soil. With intermittent waterlogging the plants developed only a few adventitious roots. Waterlogging has been shown to result in different physiological responses in barley and in other plant species. For example, higher activity levels of enzymes in the lactic acid pathway (ADH and LDH) were reported in the root of flooding sensitive barley cultivars when compared with tolerant cultivars (Wignarajah 1976; Good et al. 1989). The anaerobic stress can also induce an increase in alanine aminotransferase activity and regulate the alternative oxidase (AOX)) expression in barley roots. Leyshon et al. (1978) reported that ethylene formed in flooded soils in potentially toxic amounts at the root

surface may also play a significant role in plant damage. Differences in regulation of ionic levels (such as Na^+, K^+, Cl^- and P) of the sensitive and tolerant cultivars during waterlogging and after waterlogging (Wignarajah 1987) indicated that the sensitivity of barley to waterlogging may be associated with the inability to regulate the uptake and transport of Cl^- ions during the early stages of waterlogging stress. K^+ loss was also observed during the early stages of hypoxia (Pang et al. 2006). Root mineral coatings have also been reported to be different in different cereal species (Chenet al. 1980; Ding et al. 1995). The mineral components of root coatings were mainly composed of Fe, Mn and P, and it was reported that these elements can cause toxicity to plant roots in solution or in reduced phase, such as during waterlogging stress.

Many microscopic studies have been made on the effect of waterlogging on root morphology. Yu et al. (1969) showed that root porosities were significantly affected by various flooding treatments. Root porosities of all plants tested were higher in full-flooded treatments as compared to non-flooded ones, but no significant difference was found in barley (Yu et al. 1969). However, development of aerenchyma has been reported in a wide range of plant species when they were subjected to reduced O_2 tension. For example, extensive aerenchyma development was observed under low oxygen conditions in corn (Batten 1918), wheat (Dunn 1921), and barley (Bryan 1934; Pang 2004). Aerenchyma can be found in roots, rhizomes, stems and leaves, but are mostly common in roots. Aerenchyma formation in corn roots is preceded by the disappearance of protoplasm from the cell, bulging cells, death of cells, and by collapse of cell walls (McPherson 1939). Gunawardena et al. (2001) also showed that aerenchyma formation in plants initiated by hypoxia or ethylene appears to be a form of programmed cell death that shows characteristics in part resembling both apoptosis and cytoplasmic cell death in animal cells.

Garthwaite et al. (2003) studied the diversity in root aeration traits associated with waterlogging in the genus *Hordeum* by evaluating the growth, root aerenchyma, and the profiles of radial O_2 loss along adventitious roots in 35 wild *Hordeum* accessions and cultivated barley. Their results indicated the possibility of a link between having a barrier to radial O_2 loss and the X and H genomes in *Hordeum* species which might enable a genetic analysis of this important trait in future studies. Although there remain gaps in knowledge of the development, regulation and biochemistry of aerenchyma formation,

some scientists have investigated the possibility of introducing constitutive aerenchyma into maize (Ray et al. 1999).

2.2.2 Response of barley shoots to waterlogging stress

When soil is flooded, the dissolved oxygen in the soil water is rapidly depleted, and the shoots of barley which are sensitive to poor soil aeration suffer characteristic damage. Affected barley plants typically show conspicuous chlorosis and early death of older leaves, and slower extension of leaves (Watson et al. 1975; Drew et al. 1977; Drew 1979). Intermittent waterlogging significantly reduced the number of tillers and heads per plant of barley, reduced stem elongation (Yu et al. 1969; Drew 1979) and reduced dry weight of the barley plants (Leyshon et al. 1974). Waterlogging significantly delayed the ear emergence of barley (Leyshon et al. 1976) by 4-7 days after a 7 day flooding. Waterlogging causes a delay in the whole process of plant maturation similar to the delay in ear emergence. Stem hypertrophy is one of the symptoms associated with waterlogging in plants (Kawase 1981).

Drew et al. (1977) indicated that after barley seedlings were subjected to waterlogging stress, there was a decrease in the average concentration of nitrogen in shoots. This may due to inhibition of nitrogen uptake and the consequent redistribution of nitrogen from old leaves to younger expanding ones, resulting in the early yellowing and senescence of leaves and the retarded growth of shoots in flooded plants. Other studies also showed that although the concentration of Mn in barley was not altered by flooding, short-term flooding did reduce the concentration of N, P, and K in barley (Leyshon et al. 1974).

Flooding caused a decrease in stomatal conductance in barley plants, with a parallel decrease in transpiration and photosynthesis (Yordanova et al. 2001). It was reported that the activities of both carboxylating enzymes (RuBPC and PEPC) were differently affected by flooding. RuBPC activity was reduced when barley plants were subjected to flooding, while a significant increase in the activity of PEPC was found in all flooded plants. As a result of the inverse effect of flooding on the activity of both carboxylase enzymes, the RuBPC/PEPC ratio declined in all treated barley plants (Yordanova et al. 2001). Flooding also caused a gradual decrease in the activities of phosphoglycollate phosphatase and glycollate oxidase (Yordanova et al. 2001). A progressive decrease in chlorophyll a and b content and an increase of proline content in barley leaves was

observed. Relative to control, flooded barley plants also exhibited a large accumulation of leaf titratable acidity (Yordanova et al. 2001). The effect of alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) activity in barley seeds was investigated by comparing the behaviour of mutant and wild-type plants in flooded conditions. The authors found that there is a specific requirement for a functional ADH1 polypeptide in the immersed seed, the results indicating that the presence of ADH activity is necessary for barley to survive flooding at the germination stage (Harberd et al. 1982).

2.2.3 Effects of waterlogging stress on barley grain yield

Many studies of flooding of cereal crops have been concerned with grain yield rather than the immediate, short-term effects. Intermittent or continuous waterlogging for a short period, in most instances, seriously reduced grain yield in barley. Waterlogging affect plant yield through: reduced root growth and penetration (Belford et al. 1992); reduced production of tillers and fertile heads (Musgrave 1994; Taeb et al. 1993; Cannel et al. 1984; McDonald et al 2001); delayed ear emergence and plant maturation and reduced yield components (Waterson et al. 1976; Leyshon et al. 1978).

2.3 Mechanisms of tolerance to waterlogging in barley and other plants

To genetically understand barley waterlogging tolerance, it is important to understand the mechanisms of this stress in plants. Broadly speaking, waterlogging resistance in plants is achieved by one or more features which improve gas exchange, as well as various metabolic features which help maintain a sufficiency of energy production to sustain cell integrity and avoid irreparable damage under oxygen stress. The latter in some species extends to a limited degree of anaerobic growth to help establish contact with the atmosphere. In addition to this, response to waterlogging is often under hormonal control.

2.3.1 Tolerance involving maintaining the internal oxygen transporting system

In many plants, especially in wetland plants, an extensive oxygen transport system (aerenchyma tissue) may exist in roots, stems and leaves (Armstrong et al. 1994). This system allows a plant to transport the needed oxygen to the roots for maintaining aerobic respiration and to oxidize reducing compounds in the rhizosphere. In addition, the internal system of large gas spaces also reduces internal volume of respiring tissues and

oxygen consumption, thus, enhancing the potential for oxygen reaching the distant underground portions of the plant (Armstrong et al. 1994, 1996a, b, c). Due to such advantages, the oxygen transport system has been considered as a major mechanism critical to a plant's ability to cope with soil anaerobiosis (Kozlowski 1982, 1984a, 1984b, 1997; Drew 1990, 1992, 1997; Armstrong et al. 1991, 1994, 1996a, b, c,).

The effectiveness of gas transport is primarily dependent on two factors. The first factor is the resistance to diffusion which is proportional to root length and inversely proportional to root porosity. The Second one is the oxygen demand along the diffusion path resulting from respiratory needs as well as oxygen leakage from the roots into the rhizosphere (Luxmoore et al. 1972; Armstrong 1979). Indeed, oxygen demands of roots and rhizosphere are competitive because in flooded soils these systems compete for the plant pool of oxygen simultaneously (Armstrong and Becket 1987, Armstrong et al. 1991, 1994). As soil reduction continues, there is progressively greater demand imposed upon roots for oxygen (DeLaune et al. 1990). Literature concerning the relationship between functional aspects of gas transport within plants and soil oxidation-reduction conditions is limited. In a few studies that evaluated the relationship, it is evident that intense soil reduction promotes oxygen loss from the root to rhizosphere. For instance, a high correlation was found between radial oxygen loss rates from roots and soil Eh intensity; there was an increasingly higher oxygen loss rate as soil Eh was lessened (Kludze and DeLaune 1995)

Despite the reported increase in aerenchyma tissue formation (and hence porosity) in wetland species in response to reducing conditions, this may not be sufficient to satisfy the root respiratory needs for oxygen due to the greater radial oxygen loss rates in response to high intensity of reduction. Pezeshki et al. (1991a, 1993) found that despite a substantial enhancement of aerenchyma tissue formation, alcohol dehydrogenase activity continued to be higher in flooded than in control plants indicating continued oxygen stress in the roots of flooded plants. In addition to the effects of the intensity of reduction, differences in Eh capacity among wetland soils may influence many plant functions including oxygen transport and rhizosphere oxygenation (Kludze and DeLaune 1995).

Radial oxygen loss and the rhizosphere oxidation in flooded soils, provided that there is a gas-space continuum between root and shoot, is inevitable unless there is a critical

combination of relatively low oxygen permeability and relatively high respiratory oxygen demand in the radial path through the non-porous outer layers of the root. Thus, even roots of relatively low porosity are likely to effect some oxygenation of the rhizosphere. However, such leakages may severely curtail the length to which the roots may be aerated internally (Armstrong 1979; Armstrong & Beckett 1987) and rooting depths and exploitable soil volume might be very restricted.

As well as encouraging an aerobic microflora, radial oxygen loss to the rhizosphere can affect a variety of oxidative reactions beneficial to plants. In this respect, rhizosphere oxygenation is probably of equal importance for flood tolerance as aerenchyma development or anaerobic metabolism. The precipitation of hydrated iron oxides is the most frequently observed example of rhizosphere oxidation by roots (Armstrong et al. 1967; Armstrong et al. 1992; Green et al 1977; Laan et al. 1989; St-Cyr 1988; St-Cyr et al. 1993; Snowden et al 1993;). This helps to protect against the absorption of toxic amounts of the ferrous ion, but may also help to reduce the intake of arsenic and heavy metals such as zinc and cadmium which can become absorbed or complexed to the rhizosphere iron deposits (Laxen 1983; Otte et al. 1989, 1991). Hydrogen sulphide, a potent phytotoxin (Koch et al. 1990), may be oxidized directly, or be scavenged as the relatively insoluble ferrous sulphide (Engler and Patrick 1975, Winter and Kickuth 1989). Radial oxygen loss will also support aerobic nitrifying bacteria (Hansen and Andersen 1981; Hoffmann 1990; Buuis 1994) or aerobic nitrogen-fixing bacteria (Uckert et al. 1990) in the rhizosphere. The potential for nitrification in the rhizosphere is a major consideration underlying the current use of wetlands for the purification of domestic and agricultural effluents. The studies reviewed above all suggested that internal gas transport is very important for plants to survive long-term waterlogging stress.

2.3.2 Tolerance involving metabolic adaptation

Oxygen is vital to the main energy-providing pathway of plant cells, and the presence or absence of oxygen determines metabolic activity and energy production (Greigenberger et al. 2003; Dennis et al. 2001). Oxygen is also required in several important cellular pathways, including haemoglobin, sterol and fatty-acid biosynthesis (Greigenberger et al. 2003). Compared to animals, however, plants lack efficient systems for oxygen delivery. Flooding, waterlogging or microbial activity in the soil can rapidly lead to anoxic

conditions. ATP formation through oxidative phosphorylation is inhibited and this will impair cellular metabolism and function. Under this condition, fermentation of carbohydrates enables the plant to maintain ATP production in the absence of oxygen, albeit with a reduced energy yield. It is possible that inducing the enzymes of the glycolytic pathway and sugar metabolism amplifies the flux through this pathway, in order to compensate for this reduction in energy yield. Although the fermentation pathways are not present under conditions of normal oxygen supply, their quick *de novo* induction by low oxygen conditions suggests a role in the survival mechanism. In plants, research has mainly focussed on the presence and function of fermentation pathways as a metabolic rescue mechanism when respiration is arrested (Dennis et al. 2001). Three main fermentation pathways are active in plants during flooding: ethanol, lactic acid, and a plant-specific pathway which produces alanine from glutamate and pyruvate (Dennis et al. 2001). However, we still have little knowledge on exactly how and to what extent these pathways contribute to low oxygen stress tolerance and how the three pathways are interrelated.

Although inhibition of metabolic processes is one of the biochemical mechanisms governing adaptation of plants to hypoxia, it is probably a non-specific response of organisms to various stress influences, since cells with a lowered rate of metabolism are usually less sensitive to harmful environmental factors. However, a lower rate of respiration is not characteristic of all plants adapted to hypoxia. For example, values of oxygen uptake and carbon dioxide evolution under conditions of aeration are similar in wheat and rice seedlings (Taylor et al. 1942). Nevertheless, a decrease in the rate of root respiration in rice seedlings took place considerably more slowly than that in wheat (Taylor et al. 1942), indicating that low oxygen can also lead to an inhibition of biosynthetic activity that will result in a decrease in ATP-consumption.

Another complementary strategy to a depression of metabolism is for plants to prioritise metabolic pathways and strategies that conserve energy, and hence reduce oxygen consumption. For example, plants possess two alternative biochemical pathways for sucrose degradation to hexose phosphates, which differ in their energy costs. The breakdown of a molecule of sucrose by invertase and hexokinase requires two molecules of ATP, whereas its breakdown by sucrose synthase (SuSy) and UDP-glucose pyrophosphorylase requires only one molecule of inorganic pyrophosphate (PPi) (Stitt

1998). Under a low oxygen condition, SuSy activity increases while invertase activity declines (Zeng et al. 1999; Guglielminetti et al. 1995), with SuSy predominating as the main enzyme active in sucrose breakdown in plant roots (Richard et al. 1998).

2.3.3 Hormones and plant resistance to waterlogging

Plants exhibit a wide range of morphological and anatomical responses to waterlogging or flooding stress, some of which appear to have adaptive significance. Each of these reactions is mediated by plant hormones. Of the five major hormones (indole acetic acid, ethylene, gibberellic acid, cytokinin and abscisic acid), ethylene is the hormone most closely associated with the indication of a developmental response to waterlogging. It has been clearly demonstrated that ethylene is the principal mediator promoting the development of aerenchyma in maize as well as other plants (Jachson 1985, 1987, 1989, 1990a, 1990b). When rice is submerged in water, a principal developmental characteristic is an enhanced rate of upward extension, enabling coleoptiles and shoots to gain access to oxygen, carbon dioxide and light (Jackson et al. 1991). This occurs under the influence of ethylene, which interacts with gibberellins (Musgrave et al. 1972; Kende 1987) and Auxins (Cookson et al. 1978; Horton 1987). Auxins and gibberellins are prerequisites for ethylene action and play triggering rather than regulatory functions.

Synthesis and translocation of gibberellins and cytokinins in the roots are suppressed by root injury resulting from waterlogging (Rowe et al. 1973; Vartapetian 1978). Some studies found that reduction in leaf extension or shoot elongation has been associated with interference in the synthesis and transport of these two homones as well (Burrows and Carr 1969; Railton and Reid 1973; Reid and Crozier 1971). Cytokinin may induce the expression of the haemoglobin gene in roots, leaves and inflorescences (Hunt et al. 2001) and in barley aleurone layers (Taylor et al. 1994). Haemoglobin molecules reversely bind to oxygen, the rate at which they bind and release oxygen varies, depending on the type of haemoglobin and is a defining characteristic in their cellular function. Haemoglobins induced by hypoxic stress may have an important role in the metabolism of plants under waterlogging conditions. Abscisic acid rapidly accumulates in leaves (Hiron and Wright 1973) and may contribute to stomatal closure, inhibition of shoot growth, and early leaf senescence (Millborrow 1974).

2.4 Efforts aimed to improve waterlogging tolerance in cereal crops

Waterlogging tolerance is defined in physiological studies as survival or the maintenance of growth rates under waterlogging at different stage of development relative to non waterlogged conditions, whereas the agronomic definition of waterlogging tolerance is the maintenance of relatively high grain yields under waterlogged relative to non-waterlogged conditions (Setter 2003). The agronomic definition based on grain yields alludes to the possibility that some varieties could exist that possess a mechanism of tolerance associated with escaping from anaerobic condition through dormancy or slow growth during a stress period, and have a rapid recovery following stress. Therefore evaluation of crop varieties should consider both the physiological performance during waterlogging and recovery ability after waterlogging. Germplasm evaluation based on grain yield may be confounded because of the possibility that tolerance and recovery mechanisms only partly contributed to the grain yield after the waterlogging stress was terminated (Setter et al. 2003). This is especially the case in environments where waterlogging is for a short time, and other environmental factors or stress may also affect the grain yield. Sometimes other stresses may even contribute more to the final grain yield than waterlogging stress unless the waterlogging events are during or close to the grain filling period.

2.4.1 Genetic diversity for waterlogging tolerance in some cereal crops

Genetic differences exist for tolerance to waterlogging in wheat (Davies and Hillman 1988; Bourget et al. 1996; Thomson et al. 1992; Ding and Musgrave 1995; Huang et al. 1994a, 1994b; McKersie and Hunt 1987; Gardner and Flood 1993). For example, Huang et al. (1994) showed that there is good genetic diversity for tolerance of wheat to hypoxic solution cultures. In a glasshouse experiment with 14 wheat varieties and several doubled haploid wheat lines, Setter et al. (1999) showed that there was good diversity for waterlogging tolerance based on shoot growth during continuous waterlogging for four weeks, and after waterlogging during 3 weeks recovery period following drainage. Davis et al. (1988) demonstrated variation in vegetative growth and yield under continuous flooding of 4-week-old plants of various wheat species, with the hexaploid *Triticum macha* and the tetraploid *T. dicoccum* being the most tolerant. Inter-variety differences in wheat seedling survival after 7 days flooding combined with cold treatments have also

been reported by McKersie and Hunt (1987).

Xu et al. (1997) reported that there was an obvious difference in waterlogging tolerance among 20 different barley varieties (lines), the authors showing that some varieties including Weisubuzi, Su5078, Tong83-11, Tong 88-58 have better tolerance to waterlogging stress than others based on grain yield and yield components (Xu et al. 1997). Setter et al. (1999) demonstrated a genetic diversity of waterlogging tolerance in barley exposed to intermittent waterlogging over 4 weeks, and waterlogging tolerance was assessed using leaf chlorosis following waterlogging. According to their results, grain yield of barley was reduced by 51-84% of non-waterlogged plants, but the order of yield reduction did not coincide with that of leaf chlorosis. Yang et al. (1999) compared the waterlogging tolerance of eight barley dwarf-mutants. The results showed that physiological and biochemical characters such as green leaf number of main stem, fresh weight of plant and activity of superoxide dismutase (SOD) in the flag leaf were greatly changed by waterlogging stress, which also resulted in a decrease in grain yield. The results of that experiment also showed that there is a significant difference in tolerance to waterlogging among the mutants, and 95-39, 95-31 and 95-53 are better than others in waterlogging tolerance. Hamachi et al. (1989) reported that 8 barley parental lines and their F1 and F2 hybrids were grown under waterlogged conditions at the internode elongation stage, and selected for a reduction in numbers of dead leaves as the waterlogging tolerance indicator. Heterosis for tolerance expressed as reduction in damage was observed in F1s, and frequency distributions of damage in F2s showed continuous variation. These results indicated that screening for waterlogging tolerance by the amount of dead leaves was a useful criterion and that endurance was under polygenic control.

2.4.2 Large-scale screening for barley germplasm with good waterlogging tolerance

Extensive screening of barley germplasm for waterlogging or wet tolerance has occurred in China and Japan. Work by Qiu and Ke (1991) involved screening 4,572 varieties in Shanghai province, China. Waterlogging was imposed at three times (leaf 3 stage, stem elongation and heading) for 10-15 days each. Calculation of a "damage index" was based on yield of plants in waterlogging treatments expressed as a percentage of yield under non-waterlogged conditions. Varieties were classified into one of five grades of damage:

0.4% of varieties had less than 1% damage; 5% had 1-10% damage; 30% had 10-20% damage; 32% had 20-40% damage; the remaining 33% had over 40% damage. The majority of the 16 varieties identified with the highest waterlogging tolerance also had either very early, early or medium maturity, indicating that delayed recovery was not the mechanism of tolerance. These varieties also showed other attractive qualities such as large grain size and stiff stems (Qiu and Ke 1991).

Takeda et al. evaluated 4,096 barley varieties for tolerance to waterlogging commencing at leaf 3 stage for the entire growth duration (1987). The most tolerant varieties survived more than one month at 25 C^{o}. Of the 33 varieties they found most tolerant, 15 were Japanese varieties.

2.4.3 Heritability of waterlogging tolerance in cereal crops

Most of the early published research in genetic studies on waterlogging tolerance was done in wheat, and based on leaf chlorosis or leaf/plant death (Cao et al. 1992, 1994, 1995; Cai et al. 1996). Van Ginkel (1992) demonstrated that there is a high negative correlation between leaf chlorosis (or death) and grain yield in wheat. These researchers indicated that waterlogging tolerance is under genetic control, and is heritable, with a broad sense heritability estimated to be over 70% (Cao et al. 1992, 1994, 1995; Cai et al. 1996). The only work to evaluate the heritability of waterlogging tolerance based on plant grain yield using 20 wheat varieties is by Bao (1997). He found that heritability for tolerance to 15 days waterlogging in the field at the tillering stage and the booting stage was 74.7 and 80.2%, respectively. These authors concluded that it is possible to improve waterlogging tolerance in wheat by selecting progeny in early generations based on related traits. Cao et al. (1992, 1995) found that waterlogging tolerance based on leaf chlorosis was controlled by one dominant gene, but tolerance based on traits such as green leaves/main stem, plant height, grains per ear and 1000-grain weight could be controlled by multiple genes in the varieties involved in their study (Cao. et al. 1994).

2.4.4 Chromosomes or genes associated with waterlogging tolerance in cereal crops

In a test with inter-varietal substitution lines, Poysa (1984) found that homoeologous

group 5 chromosomes were associated with positive effects on wheat seedling survival. Taeb et al. (1993) reported that the related species *Thinopyrum elongatum* and *Elytrigia repens* had better waterlogging tolerance than wheat when comparing a number of *Triticeae* species for tiller production, shoot dry matter production and root penetration in waterlogged soil. Tests of a number of wheat-alien amphiploids showed that there was at least partial expression of this exotic genetic variation in a wheat genetic background. The presence of chromosome 2E and 4E of *Th. Elongatum* was associated with a positive effect on root growth in waterlogged conditions. The positive effect of the 4E chromosome addition was mimicked by tetrasomic lines carrying extra doses of wheat homoeologous 4B and 4D, and it was concluded that the beneficial effect contributed by the presence of 4E was probably due to an increased dosage of group 4 chromosomes. However, the positive effect of adding chromosome 2E to wheat could not be reproduced by added doses of chromosomes 2A, 2B, or 2D, suggesting that this alien chromosome carries genes for tolerance not present on its wheat homoeologues. This gene was further located to the long arm of chromosome 2E by testing ditelosomic addition lines

Boru (1996) extended the research of van Ginkel et al. (1992) by screening for waterlogging tolerance in genetic studies involving several of the tolerant wheat varieties. It was proposed that in three waterlogging tolerant wheat genotypes, tolerance was conditioned by four major genes. The three tolerant wheat genotypes used in that study carried different genes, although they all possessed one tolerant gene (*Wt1*) in common. These different genes could control different mechanisms of tolerance to waterlogging, therefore waterlogging tolerance could be substantially improved by combining all tolerance genes into one genotype (Boru et al. 1996). Based on their results of the inheritance of waterlogging tolerance in wheat by using three tolerant (Prl/Sara, Ducula and Vee/Myna) and two sensitive (Seri-82 and Kite/Glen) spring bread wheat lines, Boru (2001) proposed that the expression of waterlogging tolerance was not influenced by a maternal effect. The F1 hybrids were intermediate for leaf chlorosis, indicating that tolerance was additive. Quantitative analysis also indicated that additive gene effects mainly controlled waterlogging tolerance in these crosses. Segregation ratios of F3 lines indicated that up to four genes controlled waterlogging tolerance in these crosses, with two genes adequate to provide significant tolerance. Some of the work in China (Cao et al.

1994) also indicated that additive gene action is the major determinant of the inheritance of waterlogging tolerance.

2.4.5 Quantitative trait loci (QTL) for waterlogging tolerance identified in cereal crops

As indicated previously, plant response to waterlogging stress is quite complex, and is associated with a large number of different morphological, physiological and biochemical changes. Genetic studies of waterlogging tolerance in wheat and maize showed that some of those responses such as leaf chlorosis or leaf injury, adventitious root formation are controlled by multiple genes (Boru et al. 1996; Mano et al. 2005; Mano et al. 2006). Since the advent of molecular technologies to investigate the fundamental structure and actions of plant genomes and the beginning of an understanding of the genetic and biochemical bases of abiotic stress tolerance, some of recent studies on flooding stress form an excellent basis for modern breeding approaches. Advancement in producing stress-tolerant crop varieties may now be accelerated more rapidly than previously thought possible.

In a genetic analysis of rice, Xu and Mackill (1996) localized a major gene for submergence on chromosome 9 (*Sub1*). Nandi et al. (1997) additionally localized minor QTLs for submergence tolerance on chromosomes 6, 7, 11, and 12. Xu et al. (2000) fine-mapped *Sub1* from variety FR13A, using a very large mapping population derived from a cross between M202 and a derivative of FR13A. Two markers co-segregated with *Sub1* and others were at a distance of 0.2 cM on the genetic map of rice. Sripongpangkul et al. (2000) mapped a gene for submergence tolerance from the cultivar IR74, the gene was mapped to the same location as Sub1 and is presumably allelic with it. Toojinda et al. (2003) also identified QTLs for traits associated with submergence stress tolerance at the seedling stage in three rice mapping populations, where they found that in different years and seasons and with different mapping populations, the QTLs controlling traits related to submergence tolerance were mapped on many genomic regions. However, the consistently detected QTL_{ch9} indicated a common genetic factor controlling submergence tolerance in rice, and it is the most important submergence tolerance QTL in the three populations. This QTL (QTL_{ch9}) was mapped near the sub1 locus previously identified by Xu and Mackill (1996) and Nandi et al. (1997). In addition to the major QTL on

chromosome 9, secondary QTL that influence submergence tolerance in the three mapping populations have been located to rice chromosomes 1, 2, 5, 7, 10 and 11. Siangliw et al. (2003) investigated the potential of introgressing submergence tolerance simultaneously from three tolerant cultivars into the susceptible but otherwise highly desirable fragrant cultivar KDML 105 using back-crossing and marker assisted selection. The experiment used PCR-based markers tightly linked to the QTLs identified in Toojinda's experiment (Toojinda et al. 2003). It was demonstrated that the individuals of a BC4F3 line that retained a critical region on chromosome 9 transferred from tolerant lines were also tolerant of complete submergence while retaining all the agronomically desirable traits of KDML105. The close association between tightly linked markers of the tolerance locus on chromosome 9 and submergence tolerance in the field indicated the considerable promise of using these markers in breeding programs for selecting increased submergence tolerance.

Identification of QTLs controlling flooding tolerance in other crops was also reported recently. VanToai et al. (2001) investigated the QTLs contributing to waterlogging tolerance in soybean in two mapping populations and identified a single QTL, linked to marker Sat-064, from the tolerant cultivar Archer which was associated with improved plant growth and grain yield in waterlogged environments. Near isogenic lines with and without the sat-064 marker were developed to evaluate the effect of this QTL on waterlogging tolerance in southern environments and genetic backgrounds in USA, and assess variability for waterlogging tolerance in Archer derived populations under waterlogging conditions (Reyna et al. 2003). The results demonstrated that the Sat-064 marker did not account for a significant portion of the variability among the NILs for either visual assessment of waterlogging injury or yield-based assessment of waterlogging tolerance. Several possible reasons have been suggested by the authors for why the Archer allele at Sat-064 did not affect waterlogging tolerance in that study (Reyna et al. 2003) and it seems that improving waterlogging tolerance by using marker-assisted selection is still not available in this crop, but the feasibility could be enhanced by more efforts in the future.

A QTL for flooding tolerance based on the degree of leaf injury in maize has been located on chromosome 1 by Mano et al. (2006), and another QTL for flooding tolerance, evaluated as dry matter production under flooding stress was mapped to the same position

as the QTL that controlling leaf injury (Mano et al. 2006). These results suggested that the potential to increase productivity by transferring flooding tolerance genes from a tolerant cultivar to elite maize inbred lines, or by pyramiding QTLs controlling different sub-traits responsible for flooding tolerance such as reduction tolerance (Mano et al. 2006), adventitious root formation (Mano et al. 2005a, Mano et al. 2005 b), and aerenchyma formation (Ray et al. 1999) into a single genotype has been enhanced in this crop.

Attempts at QTL mapping for flooding tolerance have also been reported on wheat (Burgos et al. 2001; Cakir et al. 2005) and barnyard grass (*Echinochloa crus-galli*) (Fukao et al. 2004). Two QTLs were detected for waterlogging tolerance in *E. crus-galli,* and the authors tried to investigate if one of the two QTLs is closely linked to *Sub1,* a major QTL controlling submergence tolerance in rice (Fukao et al. 2004). Several rice chromosome 9 probes were applied to *Echinochloa*, but none of the probes detected polymorphism in that experiment. However, this is the first report on comparatively mapping genes or QTLs controlling flooding tolerance among closely related species. Four QTLs were identified for waterlogged shoot growth and waterlogged root growth, and these QTLs explain up to 17% and 22% of the variation in waterlogged shoot growth and waterlogged root growth, respectively. The most significant QTLs for both the two traits were located on the long arm of chromosome 7B (Cakir et al. 2005).

For barley, however, an overwhelming proportion of waterlogging studies has concentrated on morphological and physiological response to this trait, with very limited genetic studies carried out. Little progress has been made on improvement of barley varieties for waterlogging tolerance due to lack of basic genetic understanding. With the availability of a large numbers of molecular marker systems and many powerful computer programs for QTL analysis, quantitative trait locus (QTL) identification has become possible for some complex quantitative traits. The characterization of QTLs for various adaptive traits for barley waterlogging tolerance would be particularly important, and use of marker assisted selection could be the most efficient way to bring waterlogging tolerance into commercial barley varieties. To achieve the objective of improving tolerance to waterlogging in barley, this PhD project aimed to investigate the quantitative inheritance of barley waterlogging tolerance and develop molecular markers for waterlogging tolerance in barley.

Chapter 3 Quantitative inheritance of waterlogging tolerance in barley

3.1 Introduction

Soil waterlogging usually influences plant growth in a negative way. The inhibition of nitrogen uptake, and the consequent redistribution of nitrogen within the shoot are important contributory factors in the early senescence of leaves and the retarded growth of shoots in flooded plants (Drew and Sisworo, 1977). A decrease in the nitrogen concentration in shoots of barley seedlings can occur rapidly after the onset of flooding and precede leaf chlorosis (Drew and Sisworo, 1977; Wang et al. 1996) and consequently reduces shoot and root growth, dry matter accumulation and final yield (Kozlowski, 1984; Drew, 1991; Huang et al. 1994a, 1994b; Malik et al. 2002). Roots are also injured by O_2 deficiency and metabolism changes during acclimation to low concentrations of O_2 (Drew, 1997). Crops differ in their tolerance to excess soil water condition (Tokimasa, 1951). Barley is relatively susceptible to waterlogging (Wang et al. 1996). The most resistant group of barley cultivars had nearly the same waterlogging tolerance as a rather susceptible group of wheats (*Triticum* spp.) (Ikeda et al.1955). Waterlogging is estimated to reduce yields on average by 20-25%, but the loss may exceed 50% depending on the stage of plant development affected (Setter et al. 1999). Bandyopadhyay and Sen (1992) reported more than 50 per cent yield loss after 2 days and 80% yield loss after 3 days of super-saturation treatment in barley plants that were first grown for six weeks in a coastal saline soil.

Bringing tolerance of waterlogging into barley cultivars is an important breeding objective in high rainfall areas or where subsoils have low infiltration rates. To fulfil this aim, the most important step is to find waterlogging tolerance genes in barley germplasm. Takeda and Fukuyama (1986) tested 3457 cultivars (preserved at the Barley Germplasm Center, Okayama University) by submerging 50 sterilized grains of each in deionized water in a test tube for 4 days at 25°C and subsequently determining their germination percentage after 4 days on moistened filter paper at 25°C. The germination percentage ranged from 0 to 100. The collections from China, Japan and Korea contained many tolerant cultivars (average indices 71.6, 66.3, and 60.5, respectively) while those from

North Africa, Ethiopia and southwest Asia showed few tolerant cultivars (19.6, 13.8, and 13.2, respectively). The most tolerant cultivars retained complete germinability after 8 days' soaking at 25°C. Qiu and Ke (1991), after testing germination under waterlogging conditions in 4572 barley cultivars, reported that some germplasm showed a very high level of tolerance. Fufa and Assefa (1995) reported some variation among genotypes in their tolerance to waterlogging and suggested locally adapted landraces could be major sources of tolerance. Pang et al. (2005) showed that cultivars differed in their tolerance to waterlogging with some Chinese cultivars showing much better tolerance than the Australian cultivars tested.

Waterlogging tolerance is likely to be a complex trait which is related to many morphological and physiological traits that are under strong environmental influence. Direct selection on grain yield has low effectiveness since the heritability of the yield after waterlogging has been reported to be very low (Collaku and Harrison, 2005). Leaf chlorosis after waterlogging is one of the major indices used by researchers in different crops such as wheat (Ikeda et al. 1954; Cao et al. 1995; Cai et al. 1996; Boru et al. 2001), soybean (Reyna et al. 2003) and barley (Hamachi et al. 1990). Understanding the genetic behaviour of waterlogging tolerance is also important for breeding cultivars with waterlogging tolerance. Boru et al. (2001) studied the genetic behaviour of waterlogging tolerance in wheat using leaf chlorosis as the indicator of the tolerance and found that the tolerance was mainly controlled by additive gene effects. The pre-germination flooding tolerance of sorghum seed was also found to be controlled mainly by additive genes and the heritability was high in both broad (0.97) and narrow (0.75) sense, indicating that selection for tolerance could be effective in early generations (Thseng and Hou, 1993). Different results were found in wheat (Cao et al. 1995), Makha wheat *Triticum macha* (Fang et al. 1997) and maize (*Zea mays ssp. mays*) (Sachs, 1993), indicating that waterlogging tolerance was controlled by one dominant gene. The broad sense heritability of waterlogging tolerance of wheat ranged from as high as 0.71 (Cai et al. 1996) and 0.74 (Bao, 1997) to as low as 0.25 (Collaku and Harrison, 2005), depending on the genetic material and the testing method used.

There are still few reports on the heritability of, and gene effects on barley waterlogging tolerance. The realised heritability for flooding tolerance of barley in 4 F_4-F_6 populations ranged from 0.12 to 0.48, based on percentage of dead leaf. Realised heritability estimates

for 3 of the crosses ranged from -0.02 to 1.06 and from -0.12 to 0.32 on the basis of the tolerance index of culm length and grain yield, respectively (Hamachi et al. 1990). In the experiment reported here, six cultivars with different levels of waterlogging tolerance were selected to make crosses in a half diallel pattern to study the genetic control and general and specific combining abilities for waterlogging tolerance. Further information was sought from a doubled haploid population between the two cultivars with the largest difference in waterlogging tolerance.

3.2 Materials and methods

3.2.1 Cultivars (or crosses) and waterlogging treatment

Experiment 1. Six barley varieties, TX9425, YYXT, DYSYH (all Chinese cultivars), Franklin, Gairdner (both Australian cultivars) and Naso Nijo (Japanese cultivar) were selected and crossed in all possible combinations but without reciprocal crosses. The three Chinese cultivars had much better waterlogging tolerance than the other cultivars (Zhou et al. 2003: Pang et al. 2005). The Chinese and Japanese cultivars have earlier maturity than the Australian cultivars. The six parents and the 15 F_2 populations were sown in stainless steel tanks (200 cm x 100 cm x 85 cm) filled with soil from Cressy Research Station, Tasmania, Australia (where waterlogging occurs regularly) during the 2003/04 summer at Mt Pleasant Laboratories in Launceston, Tasmania. Each cultivar or F_2 population contained 10 to 12 plants. Two replications (tanks) were used. Starting from the three-leaf stage, all the cultivars and F_2s were subjected to waterlogging (keeping the water level just above the soil surface) for ten days until severe damage occurred in susceptible plants. The percentage of yellow leaf area of each plant was recorded immediately after the termination of waterlogging. The average value of the 10 to 12 plants of each cultivar or F_2 population was used in the final analysis.

Experiment 2. Similar waterlogging treatments were conducted in 1.5-L pots (filled with similar soil) for all the parents and F_2 populations during the 2005/06 summer in a glasshouse. Each pot contained 15 plants and three replications were applied. The pots were placed in 40-L bins to obtain similar waterlogging conditions. The waterlogging treatment was the same as that described above, starting from three-leaf stage and ending when severe damage was shown in susceptible cultivars.

Experiment 3. A further experiment compared 350 doubled haploid linese (DHs) derived from the cross between TX9425 and Naso Nijo using isolated microspore culture (Davies, 2003). Five seeds of each DH line were sown in each pot and two replications were used, all the pots were put into a large waterlogging facility (Fig. 3.1) at Mt Pleasant Laboratories in Launceston, Tasmania, Australia with the parents as control during the 2004/05 summer. The experiment design consisted of two randomized blocks. The same waterlogging treatment was applied as in experiments 1 and 2.

Fig. 3.1 Facilities used for waterlogging experiment

3.2.2 Statistical analysis

Parental lines and F_2s were subjected to an analysis of variance using SAS procedure Proc ANOVA. The validity of the additive-dominance model was assessed using joint regression covariance/variance (Wr/Vr) analysis, and analysis of variance of (Wr + Vr) and (Wr – Vr) arrays according to Mather and Jinks (1977). Combining ability effects were analysed according to Griffing (1956), method 2 (½ $p(p+1)$) with a fixed model. Broad sense heritability was calculated by dividing genotypic variances by total variances and narrow sense heritability was calculated by dividing additive genetic variances by total variances. Average values of five plants of each doubled-haploid (DH) line were used to study the distribution pattern of waterlogging tolerance of the DH population. Broad sense heritability of this population was also calculated.

3.3 Results

3.3.1 Difference in waterlogging tolerance of selected parents

In this experiment, waterlogging caused significant chlorosis of the older leaves of all the cultivars. Cultivars showed significant differential tolerance to waterlogging (Table 3.2; Table 3.2). The three Chinese cultivars, TX9425, DYSYH and YYXT showed significantly lower yellow leaf percentage than Franklin, Gairdner and Naso Nijo. Figure 3.1 shows the differences between the tolerant and susceptible cultivars after waterlogging treatment. The tolerant cultivars TX9425, YYXT and DYXYH not only had less yellow leaf and healthier plants but also developed a better root system.

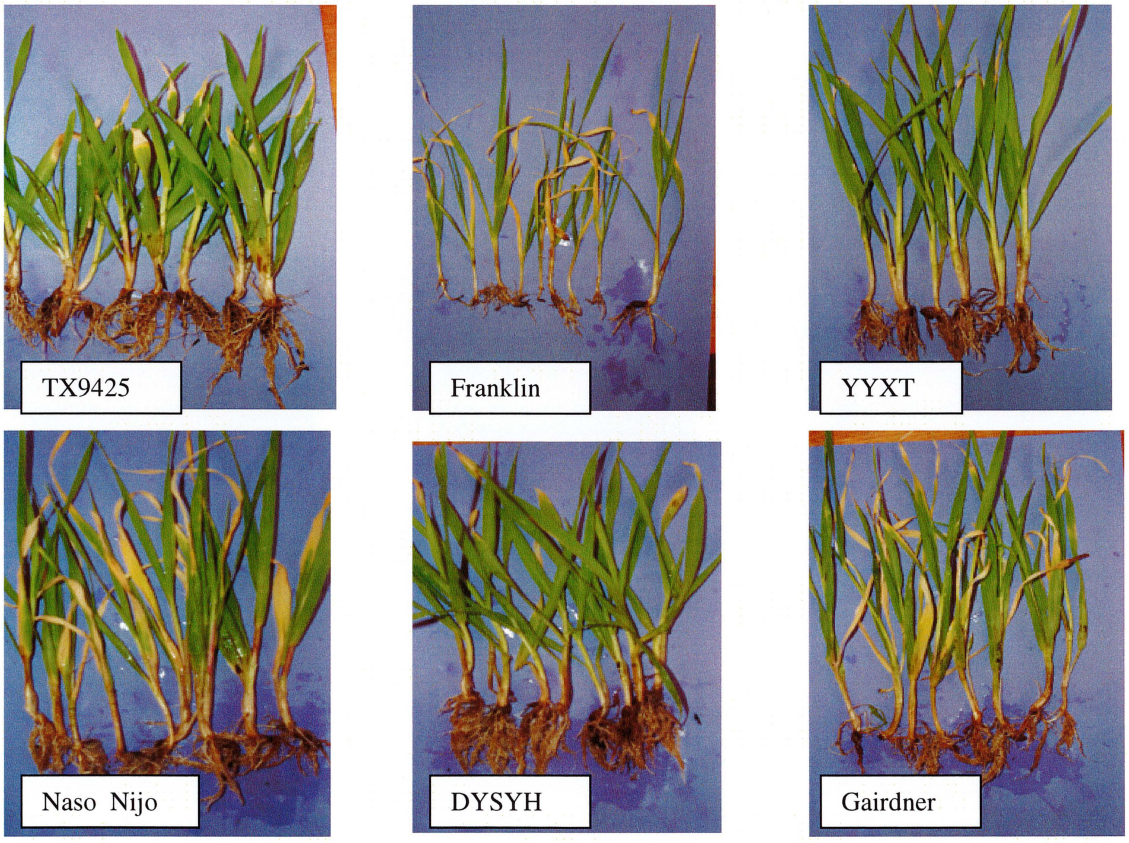


Fig. 3.2 Performance of the six selected barley varieties based on leaf chlorosis after waterlogging. The three Chinese cultivars TX9425, YYXT, DYSYH showed much better tolerance than Franklin, Gairdner (Australian cultivars) and Naso Nijo (Japanese cultivar).

Table 3.1 Half diallel data of yellow leaf percentage after waterlogging (LSD0.05 = 9.0).

	Experiment 1	Experiment 2	Average
TX9425	12.7	25.9	19.3
TX9425/Naso Nijo	31.2	32.6	31.9
TX9425/Franklin	25.6	33.7	29.6
TX9425/Gairdner	24.1	31.4	27.7
TX9425/YYXT	15.5	34.0	24.8
TX9425/DYSYH	8.9	25.7	17.3
Naso Nijo	38.0	53.0	45.5
Naso Nijo/Franklin	41.8	40.5	41.1
Naso Nijo/Gairdner	33.0	47.6	40.3
Naso Nijo/YYXT	27.6	39.1	33.3
Naso Nijo/DYSYH	23.3	40.0	31.7
Franklin	43.9	45.9	44.9
Franklin/Gairdner	39.6	44.1	41.9
Franklin/YYXT	29.9	36.9	33.4
Franklin/DYSYH	23.3	37.4	30.3
Gairdner	31.3	43.3	37.3
Gairdner/YYXT	20.4	38.4	29.4
Gairdner/DYSYH	15.6	35.8	25.7
YYXT	7.0	34.2	20.6
YYXT/DYSYH	6.9	28.3	17.6
DYSYH	5.0	25.2	15.1

Growing conditions also had significant effects on yellow leaf percentage during waterlogging treatment. Even though interactions between cultivar and environment were not significant, the relative differences between cultivars were much less in Experiment 2, the pot experiment. The yellow leaf percentages (Table 3.1) of the tolerant parent cultivars were from 5.0% to 12.7% in experiment 1 and from 25.2% to 34.2% in experiment 2 while for susceptible cultivars, the ranges were from 31.3% to 43.9% in experiment 1 and from 43.3% to 53.0% in Experiment 2. Thus, to make an effective

evaluation of waterlogging tolerance, it is important to provide suitable conditions where differences are highlighted.

3.3.2 Diallel analysis

In the crosses between waterlogging tolerant cultivars and susceptible cultivars, F_2 populations showed extensive segregation in yellow leaf percentage. For example, the yellow leaf percentage of the F_2 from the Franklin × YYXT cross ranged from 5 to 80%. Tolerant cultivars and crosses among them showed less variation between individuals. The yellow leaf percentage of DYSYH, YYXT and their F_2 population all ranged from 5 to 10%. The average value of yellow leaf percentage of all the parents and F_2 populations after waterlogging is shown in Table 3.1. Variances (Vr) and covariances (Wr) of each array in the diallel table were calculated and Wr was plotted against Vr (Figure 3.3).

As shown in Table 3.2, significant differences in waterlogging tolerance were found between varieties, indicating significant genetic variation. In Figure 3.3, the regression coefficient of Wr on Vr is 1.2385 which does not differ significantly from 1, indicating that there is no evidence of non-allelic interaction (epistasis). Thus the additive-dominance model is adequate to account for the behaviour of waterlogging tolerance involved in these varieties. The lowest point is from the Naso Nijo array, indicating that Naso Nijo had the largest number of dominant alleles (for waterlogging susceptibility), while the highest is from the YYXT array which carried the smallest number of dominant alleles (for waterlogging tolerance). However, as shown in Table 3.3, due to the relatively small dominance effects, especially in the average value of F_2 populations with only half of the dominance effect expressed, the variance (Vr) for each array was not significantly different, indicating that there were no significant dominant effects for this trait. The difference among covariances (Wr) of the family means within the array with the phenotypes of their respective non-recurrent parents was not significant again, and indicated that statistical analysis failed to detect any dominance effect in this experiment. This conclusion was confirmed by Wr+Vr and Wr-Vr analysis (Table 3.4). For both of Wr+Vr and Wr-Vr, there were no significant differences between arrays, indicating no significant dominance or non-allelic interaction.

Table 3.2 ANOVA of waterlogging tolerance (yellow leaf percentage) of the six parents.

Source of Variation	df	SS	MS	F
Varieties	5	2923.68	584.74	26.43***
Replication	1	21.28	21.28	0.96
Error	5	110.63	22.13	

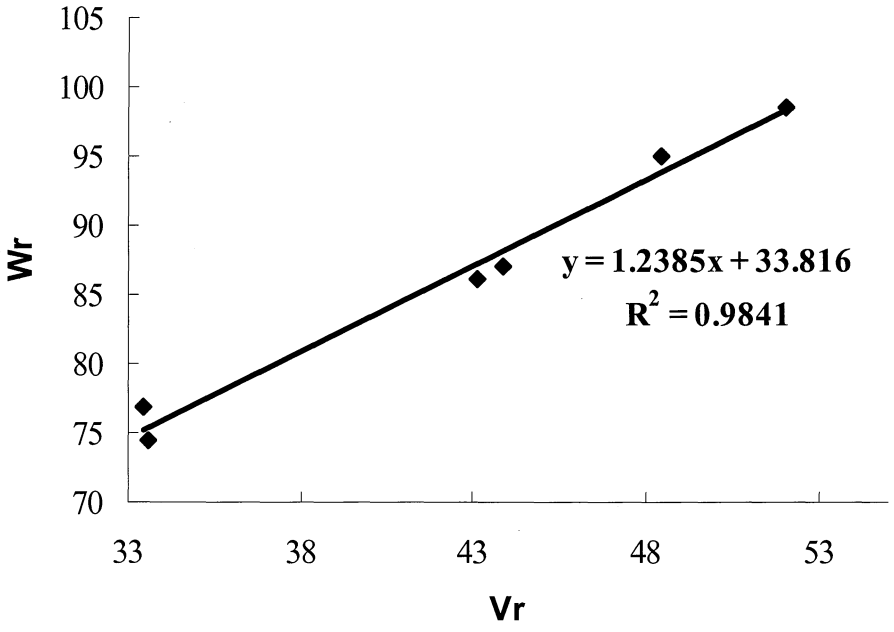


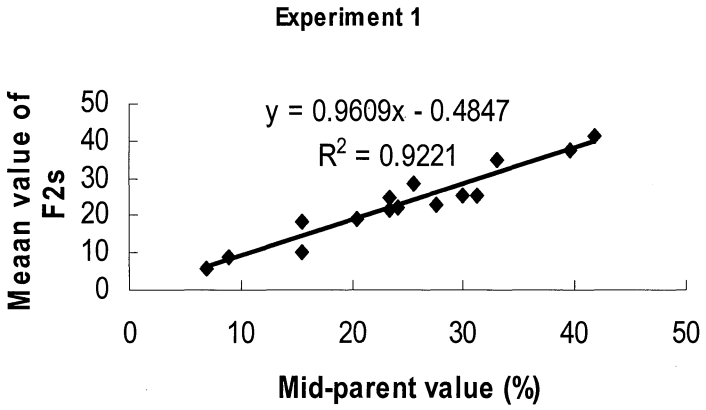
Fig. 3.3 The Wr/Vr graph for leaf chlorosis after waterlogging. Vr is the array variances, Wr is the covariance of the family means within the array with the phenotypes of their respective non-recurrent parents. The slope of the regression line is 1.2385, which does not differ significantly from 1

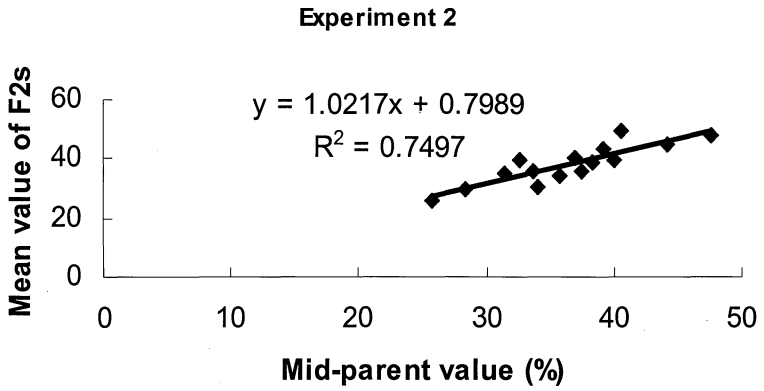
Table 3.3 Half diallel data of yellow leaf percentage after waterlogging, showing the results of Vr and Wr calculation

	DYSYH	TX9425	YYXT	Gairdner	Franklin	Naso Nijo	Mean	Vr	Wr
DYSYH	15.1	17.3	17.6	25.7	30.3	31.7	22.95	52.06	98.59
TX9425		19.3	24.8	27.7	29.6	31.9	25.1	33.56	74.51
YYXT			20.6	29.4	33.4	33.3	26.52	43.83	87.12
Gairdner				37.3	41.9	40.3	33.72	48.45	94.91
Franklin					44.9	41.1	36.87	43.15	86.05
Naso Nijo						45.5	37.3	33.44	76.89

Table 3.4 ANOVA of the effects of waterlogging on the percentage of yellow leaves per plant

	Item	df	SS	MS	F
Wr+Vr	Between arrays	5	15368.5	3073.7	0.25
	Within arrays	6	71641.9	11940.3	
Wr-Vr	Between arrays	5	404.4	80.8	0.22
	Within arrays	6	2173.6	362.3	





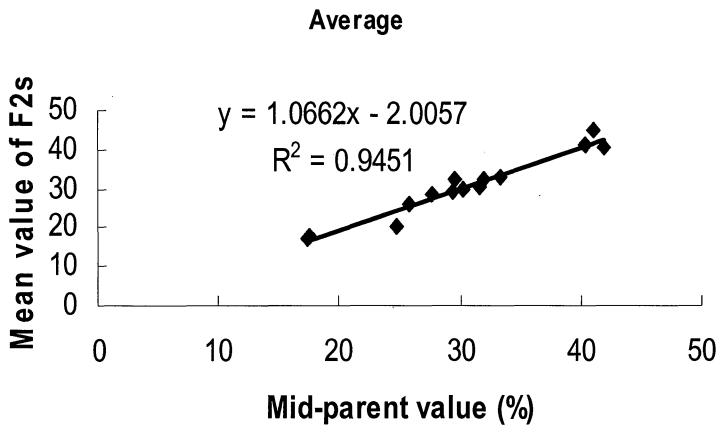


Fig. 3.4 Correlations between average yellow leaf percentages of F_2 populations and those of the mid-parent values.

3.3.3 Combining ability

Table 3.1 lists the yellow leaf percentages of parents and their F_2 populations after waterlogging treatment from both Experiments 1 and 2. ANOVA showed that even though growing conditions had very great effects, significant differences were found between different parent or F_2 populations ($P < 0.01$). The interactions between cultivar/F_2 and growing conditions were relatively small and the overall ranking of the cultivars or F_2 populations in yellow leaf percentage changed little between experiments.

The variance of general combining ability (GCA) was highly significant ($P < 0.01$) and that of specific combining ability (SCA) was not significant (Table 3.5), indicating that waterlogging tolerance was mainly controlled by additive effects and that no significant dominance effect or non-allelic interaction could be detected. Of all the cultivars, DYSYH had the lowest negative GCA (-7.5, lowest yellow leaf percentage), and therefore showed greater tolerance than the other two tolerant cultivars, YYXT (-4.1) and TX9425 (-5.4) (Table 3.6). The other three cultivars showed positive GCA (higher yellow leaf percentage after waterlogging). Franklin and Naso Nijo have been reported as the most susceptible parents in regard to waterlogging tolerance in other work in our laboratory (Pang et al. 2004; Pang et al. 2005).

Significant correlations ($R^2 = 0.92$ for experiment 1 and $R^2 = 0.75$ for experiment 2) were found between yellow leaf percentage of hybrids (F_2s) and that of mid-parents (Figure 3.4). The average yellow leaf percentages of all the crosses were similar to the mid-parent value, confirming that the tolerance was mainly controlled by additive effects. Since no significant dominant effect on waterlogging tolerance was found in these experiments, the estimated broad-sense heritability (h^2_B) was the same as narrow-sense heritability (h^2_N). From the variance of GCA, SCA and experimental error, the estimated heritability ($h^2_B = h^2_N$) was 0.73. The estimation was based on the average value of different populations. When the estimation was based on individual experiments, the broad sense heritability was 0.85 for Experiment 1 and 0.58 for Experiment 2. A lower value of heritability would be expected if the estimation had been based on single plants.

Table 3.5 ANOVA of combining ability in waterlogging tolerance (yellow leaf percentage)

Source of variance	DF	SS	MS	F
GCA	5	2752.57	550.51	60.434***
SCA	14	89.9211	5.9947	0.6581
Error	19	/	9.1094	

Table 3.6 GCA of waterlogging tolerance (yellow leaf percentage) of parents and F_2s.

Parents	Naso Nijo	Franklin	Gairdner	TX9425	YYXT	DYSYH
GCA	7.06	6.66	3.33	-5.38	-4.15	-7.52

SE=1.31; $t_{(63,0.05)}$ =2.00; $t_{(20,0.01)}$ =2.66

3.3.4 Segregation of DH population between tolerant and susceptible cultivars

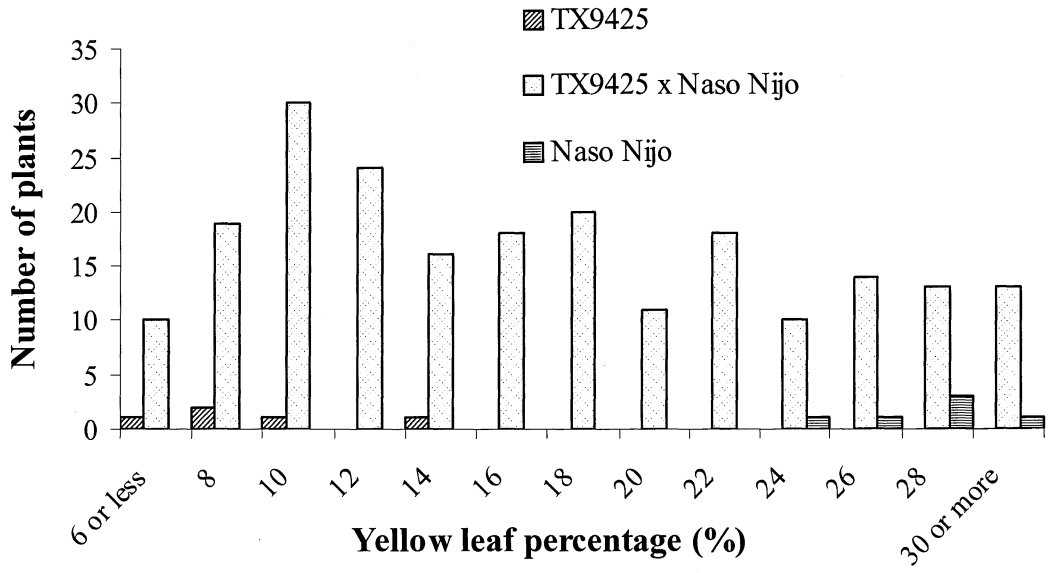


Fig. 3.5 Distribution of waterlogging tolerance of the DH lines from the cross between TX9425 and Naso Nijo

Figure 3.5 shows the distribution of waterlogging tolerance of the DH lines from the cross between TX9425 and Naso Nijo. The average yellow leaf percentages were 7.9 for TX9425, 26.7 for Naso Nijo and 16.0 for the DH population. The tolerance of the DH lines showed continuous distribution, ranging from very tolerant to very susceptible. While there was a good proportion of lines in the tolerant class, and hence scope for further selection, there was no evidence of bimodal distribution and hence of single gene effects.

3.4 Discussion

Waterlogging inhibits the uptake of nitrogen which leads to the decrease of nitrogen concentration in shoots of barley seedlings (Drew and Sisworo, 1977). Pang et al. (2005) found that both shoot and root growth was negatively affected by waterlogging. As waterlogging stress developed, chlorophyll content, CO_2 assimilation rate, and maximal quantum efficiency of photosystem II (Fv/Fm, Fv: maximum fluorescence; Fm: difference between the maximum and minimum fluorescence) decreased significantly, with cultivars showing less yellow leaf percentage having less adverse effects (Pang et al. 2005). Dead leaf percentage under excess soil moisture was thought by Hamachi et al (1990) to be the best criterion for selection for flooding tolerance in early generations because its heritability values are relatively constant, it is easy to measure and it was correlated with reduction of grain yield/plant and culm length (Hamachi et al. 1989). Oxygen deficiency in the rooting zone occurs under waterlogging conditions. The lack of oxygen can severely damage the root (Drew, 1997). Figure 3.1 showed that the tolerant cultivar not only had less yellow leaf and healthier plants but developed a better root system, which is consistent with the previous report (Pang et al. 2005). The three Chinese cultivars used in this experiment all showed very good waterlogging tolerance with significantly lower yellow leaf percentage than other cultivars. The tolerance may also be partly contributed by the formation of aerenchyma in roots under waterlogging conditions. For example, aerenchyma accounted for 23.9% and 7.1% of the root cross-section area for TX9425 and Naso Nijo, respectively, after three weeks waterlogging (Pang et al. 2005). Preliminary yield trials (data not shown) showed that under waterlogging conditions, the yield reductions of Franklin and TX9425 were 86% and 28% in a pot experiment and 61% and 39% in a controlled field experiment.

The genetic behaviour of waterlogging tolerance followed an additive-dominance model and no significant dominance effects were found in this study. The mean yellow leaf percentages of all the F_2s were similar to that of their mid-parent value. The results were similar to those previously reported in wheat (Boru et al. 2001) and sorghum (*Sorghum vulgare*) (Thseng and Hou, 1993), both showing that the waterlogging tolerance was mainly controlled by additive gene effects. However, Cao et al. (1992, 1995) found that the waterlogging tolerance of a wheat cultivar was controlled by a single dominant gene. Hamachi et al. (1989) also reported dominance effects on waterlogging tolerance in barley. The continuous distribution of waterlogging tolerance in a doubled haploid population generated from a cross of TX9425 (tolerant) and Naso Nijo (susceptible) indicated that the tolerance was likely to be controlled by several genes, which is consistent with the earlier report by Hamachi et al (1989) but different from the results in wheat (Cao et al. 1992, 1995), in which a single gene was involved in waterlogging tolerance.

The high heritability and the presence of only additive effects for waterlogging tolerance indicated that selecting in early generations for this trait would be effective. High heritability of waterlogging tolerance was also reported in sorghum (Thseng and Hou, 1993) and wheat (Cai et al. 1996) but in each case, the existence of dominance effects was reported. In the current study, the estimation of the heritability was based on the average values of different populations. If the estimation of the heritability is based on single plants, the value could be much lower since greater variation was observed even within a homozygous population (parental cultivar). With the DH population from TX9425/Naso Nijo, the h^2_B estimated from the average values (0.88) was much higher than that from individual plants (0.65). Even with different experimental conditions the evaluated heritability may differ greatly. In this experiment, the h^2_B was 0.85 for Experiment 1 but only 0.58 for Experiment 2. Thus it is not surprising that some earlier studies on barley showed low heritability of waterlogging tolerance (Hamachi et al. 1989, 1990).

There have been no previous reports on the combining ability of waterlogging tolerance of barley. In wheat, Cao et al. (1994) found a significant effect of GCA for the number of green leaves per main shoot after waterlogging treatment at the booting stage. They also found significant SCA effects, indicating the existence of dominance effects. In experiments reported here, there were significant GCA effects for yellow leaf percentage

after waterlogging treatment, but SCA effects were not significant, indicating that waterlogging tolerance was mainly controlled by additive gene effects. Based on these results, the selection of parent cultivars should therefore just be based on the parental estimates for waterlogging tolerance, without considering the specific combining ability. All three Chinese cultivars used in this experiment showed negative GCA (low yellow leaf percentage). Of these 3 cultivars, DYSYH is a six-rowed barley with relatively poorer agronomic traits and both YYXT and TX9425 are two-rowed and showed better agronomic traits. Thus the latter two cultivars could be more suitable for a breeding program focused on producing two-row malting barley, even though they had slightly higher yellow leaf percentage than DYSYH.

Accurate phenotyping is one of the vital criteria required for the improvement of waterlogging tolerance (Setter and Waters, 2003). In these experiments, the environment used to induce waterlogging showed very significant effects on yellow leaf percentage. The differences between tolerant and susceptible cultivars were much less in the pots (Experiment 2) than in tanks (Experiment 1). Thus, carefully chosen waterlogging conditions could make the selection much more effective. In the tank (Experiment 1), a very small variation in yellow leaf percentage was found among progeny of crosses between tolerant cultivars, indicating that the same or similar genes are probably involved in each tolerant cultivar. The yellow leaf percentage ranges for three tolerant cultivars in Experiment 1 were from 5 to 20%. In contrast, the range of yellow leaf percentage for susceptible cultivars was much greater among individuals. For example, the yellow leaf in Franklin ranged from 25 to 90%, even though most of the individuals had 30 to 40%, which may be due to variation in plant development stage when the treatment was imposed. The variation in yellow leaf percentage of different cultivars indicated that tolerant cultivars will normally not have any very susceptible individuals, whereas susceptible cultivars could have a few individuals showing better tolerance, presumably due to environmental effects rather than genetic differences within homozygous lines. Thus, when selecting individuals from an F_2 population, plants with severe leaf chlorosis can be discarded since they should almost always be susceptible. There may be a small number of apparently tolerant plants which may not contain tolerant genes. For these tolerant plants, further evaluation in F_3 is necessary.

In conclusion, general combining ability was very high for waterlogging tolerance while

no significant specific combining ability existed. Since heritability was relatively high for waterlogging tolerance, early generation selection could be efficient, especially when selections were based on the average value of the population. Well-controlled waterlogging conditions are crucial for the evaluation of this trait. Development of molecular markers could avoid environmental effects. This will be examined in subsequent chapters.

Chapter 4 Construction of linkage maps in two different barley crosses with SSR, AFLP and DArT markers

4.1 Introduction

4.1.1 DNA markers and their applications in barley genome mapping

Numerous DNA-based genetic marker analysis methods have been developed over the last two decades and used in the construction of linkage maps in barley. These include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP), single nucleotide polymorphism (SNP), and a new high-throughput marker system – Diversity array technology (DArT). Compared to the lately developed molecular markers, RFLPs have some limitations, such as low frequency of polymorphism in cereal crops, requirements for large amounts of DNA, high running cost, and are also considered to be time-consuming and labour-intensive (Gupta et al. 1999). Similar to RFLPs, RAPDs have also been put to limited use, partly owing to the low level of polymorphism detected and sometimes also partly owing to lack of reproducibility of results (Penner et al. 1993; Jones 1997; Gupta et al. 1999). Significant efforts towards large-scale characterization of SNPs were first initiated in human genome research. SNPs have since been shown to be the most common type of genetic variation in organisms and various techniques have been invented for genotyping SNP on large scale. However, discovering sequence polymorphism in non-model species is still difficult, which is particularly true for many crops including barley with limited resources and often complex genomes (Wenzl et al. 2004).

4.1.1.1 Microsatellites or Simple Sequence Repeat (SSR)

Microsatellites are simple sequence repeats (SSRs) of only a few base pairs (1-6). They are ubiquitous in eukaryotic genomes and their study has been greatly facilitated by recent advances in PCR technology. SSRs are ideal DNA markers for genetic mapping and population studies because of their abundance (Weber 1990), high level of polymorphism (Cregan et al. 1994, Saghai Maroof et al. 1994), wide dispersion in diverse

genomes (Wang et al. 1994), ease of assay by the polymerase chain reaction (PCR), and ease of dissemination among laboratories. Identification and characterization of SSRs, based on screening DNA libraries and/or searching public databases, have been reported for a number of plant species (Akkaya et al. 1992, Morgante and Olivieri 1993, Wu and Tanksley 1993, Zhao and Kochert 1993, Morgante et al. 1994, Liu et al. 1995). The utility of SSR markers has already been demonstrated in several genetic studies. These include the linkage with a virus resistance gene in soybean (Yu et al. 1994), the identification of chromosomal regions with significant effects on yield in rice (Zhang et al. 1994), and in the germplasm assessment of rice and soybean (Yang et al. 1994, Maughan et al. 1995, Rongwen et al. 1995). In a survey of 207 accessions of wild and cultivated barley (Saghai Maroof et al. 1994) as many as 37 alleles were observed at a single SSR locus. The high level of allelic diversity displays the great potential of SSR markers for the genetic mapping of barley.

The development of SSR markers for barley has followed a common pattern with the first few derived from sequences held in public databases (Saghai Maroofet al.1994, Becker and Heun 1995). This has been followed by the screening of small insert genomic libraries for SSR motifs (Liu et al. 1996, Struss and Plieske 1998). A number of approaches have also been described (Ostrander et al.1992; Edwards et al.1996). Ramsay et al. (2000) first developed 568 new SSR primer pairs for barley using this strategy. These, together with the 64 already published (Becker and Heun 1995, Liu et al. 1996, Petersen and Seberg 1998, Struss and Plieske 1998), mean that there are now 632 barley SSRs in the public domain. All these SSRs provide a considerable technological resource, providing barley breeders and geneticists with an array of suitable tools for a range of applications.

Gamsay et al. (2000) constructed a simple sequence repeat-based linkage map of barley using 242 SSRs in a single doubled-haploid population derived from the F_1 of an interspecific cross between the cultivar Lina and *Hordeum spontaneum* cv.Canada Park. Centromeric clustering of markers was observed in the linkage map. This may have been due to the non-random physical distribution of SSRs caused by an association with retroelements (Ramsay et al. 1999) or the preferential selection of longer SSRs (Areshchenkova and Ganal 1999) during SSR marker development. While this possibility cannot be discounted, the observed genetic distributions may also have been influenced

by the distribution of recombination events in the mapping population. Indeed, the strong clustering may have been exaggerated given the interspecific nature of the mapping population. Differences in the distribution and number of chiasmata in wide crosses can affect the distribution of mapped marker loci (Messeguer et al. 1991). This is supported by comparisons of the genetic distance found between common SSRs in the centromeric regions of chromosomes 4H and 6H in the Lina X *Hordeum spontaneum* map presented by Ramsay et al. (2000) and the Steptoe X Morex map presented by Liu et al. (1996). While the clustering is probably accentuated in the wide cross used, it is probable that the observed distribution reflects the basic uneven physical distribution of recombination known to occur in barley and other plants with a large genome. Considerable restriction of crossing over in the centromeric regions has been observed in barley through the use of translocation stocks (Kunzel et al. 2000).

The availability of extensive molecular maps of microsatellites, should open new avenues for tagging genes of economic importance, not only for marker-assisted selection, but also for cloning genes leading to the development of transgenic plants for crop improvement. In barley, microsatellites have been used to tag genes or QTLs. For example, Scheurer et al. (2001) identified two QTLs for relative grain yield per plant when they researched tolerance to a German strain of the PAV serotype of barley yellow dwarf virus (BYDV-PAV) in barley using skeleton maps constructed using SSRs, AFLPs and RAPDs, these two QTL could explain 47% of the phenotypic variance, and were located at 2HL (L stands for the long arm of the numbered chromosome, and H for *Hordeum*) and 3HL respectively.

The locus conferring aluminium (Al) tolerance (*Alp*) in Dayton (one of the most tolerant barley genotypes to Al) has been mapped to chromosome 4H using RFLP markers (Tang et al. 2000). However, RFLP markers are very costly, laborious and involve the use of radioisotopes and hence are not suitable for routine marker assisted selection (MAS). To increase selection efficiency for Al tolerance, Raman et al. (2001) used an F2 population of a Dayton/Harlan hybrid to map a number of PCR based microsatellite markers closely linked with the *Alp* locus. Their results showed that the *Alp* locus was flanked by several microsatellite loci. The microsatellite markers were validated using two F_2 populations derived from Dayton/Kearney and Dayton/F6ant-28. The marker correctly predicted Al tolerance with 90% accuracy in the latter cross. It was suggested that these markers could

be used to design crosses aiming to introgress Al tolerance and develop strategies for marker assisted selection (MAS).

Given the continuing rapid development of barley SSRs, it is envisaged that their development will ultimately supersede RFLPs as a means of mapping, aligning maps, and integrating different genetic studies within *Hordeum*. Their application in both linkage and diversity studies will provide a common reference that will facilitate the rapid integration of mapping data from different populations with that from ecological and biodiversity studies in barley (Ramsay et al. 2000).

4.1.1.2 Amplified Fragment Length Polymorphism (AFLP)

AFLP technology is based on selective PCR amplification of restriction fragments generated by specific restriction enzymes. In this technique, special double-stranded DNA adapters are ligated to the DNA restriction fragments (Vos et al. 1995), so that the sequences of adapters and the adjacent restriction sites serve as primer-binding sites. The primers are designed to contain the sequences that are complementary to those of adapters and the restriction sites, along with one to three selective bases added to their 3' ends. The use of selective bases allows amplification of only a subset of the restriction fragments, which still generate a large number of bands facilitating the detection of polymorphism. A comparison of different mapping techniques - RFLP, RAPD and AFLP –for their relative efficiency in detecting polymorphism demonstrated that AFLP is the most efficient (Powell et al. 1996, Lin et al. 1996, Ma and Lapitan 1998). A single primer combination detected up to eight times more polymorphism than a polymorphic RFLP marker. Thus, AFLP detected up to 16 times more loci, assuming that in barley, as in other crops, most AFLP markers are dominant as against the co-dominant nature of RFLP markers (Mackill et al. 1996, Maughan et al. 1996). The notable advantage of AFLP is its capacity to analyse a large number of polymorphic loci simultaneously throughout the genome with a single gel without prior sequence knowledge. In contrast to RAPD, AFLP is highly reproducible and also transferable between different populations (Jones 1997b; Yin et al. 1999; Waugh et al. 1997; Li et al. 1998; Rouppe van der Voort et al. 1997)

AFLP markers have been used in crop plants, including barley (Becker et al. 1995; Powell et al.1997; Waugh et al. 1997; Qi et al. 1998; Castigliani et al. 1998), rice (Mackill et al. 1996; Maheswaran et al. 1997; Powell et al. 1997; Virk et al. 1998), bread wheat

(Barrett and Kidwell 1998; Barrett et al. 1998; Goodwin et al. 1998; Koebner et al. 1998; Ma and Lapitan 1998; Bohn et al. 1999; Shan et al. 1999), Bermuda grass (Zhang et al. 1999), tomato (Thomas et al.1995), potato (van Eck et al.1995), sugar beet (Hansen et al.1998), and soybean (Maughan et al.1996, Powell et al.1996).

Yin et al. (1999) constructed an amplified fragment length polymorphism (AFLP) map covering 965 cM using 94 recombinant inbred lines of a cross between the spring barley varieties Prisma and Apex, and used the map to identify quantitative trait loci (QTLs) controlling plant height, yield and yield-determining physiological characters using an approximate multiple-QTL model, the MOM method. Toojinda et al (2000) constructed a 99-marker linkage map and mapped qualitatively inherited resistance to leaf rust and determinants of quantitative resistance to stripe rust and barley yellow dwarf virus (BYDV). Mano et al. (2001) devised a simple AFLP system consisting of small slab gels, a discontinuous buffer system, and silver staining. Using this system, they developed a barley map with 227 AFLP fragments, which were integrated with 40 previously characterised sequence-tagged sites, 3 isozymes, and 2 morphological markers to construct an integrated map. Some researchers reported that by adding AFLP markers to RFLP maps there was an increase in the map length in rice (from 1811 to 3085 cM, Maheswaran et al. 1997), sorghum (from 1352 to 1899 cM, Boivin et al. 1999) and barley (from 1096 to 2673cM, Castiglioni et al. 1998). However, according to the results of Mana et al. (2001), there was no significant extension of map length when AFLP markers were added between STS loci in barley.

The AFLP approach is now therefore widely used for developing polymorphic markers. The high frequency of identifiable AFLPs coupled with high reproducibility makes this technology an attractive tool for detecting polymorphism and determining linkages by analysing individuals from a segregating population. However, AFLP are difficult to use across pedigrees and have not been used to build a consensus map in barley. Because of this attempts have been made to convert AFLP into markers that can more easily be used such as SCARs. However, problems have been encountered while converting polymorphic AFLP bands into SCARs, because of the presence of a mixture of DNA fragments of the same size among individual bands. In a recent study in wheat and barley 26 chromosome-specific AFLP fragments were sequenced to design sequence-specific PCR primers (SCARs/STSs), only six of them gave the expected chromosome-specific

products, thus confirming that conversion of AFLP markers into sequence-specific SCARs/STSs is not easy (Shan et al.1999). This will limits the utility of AFLP markers in barley breeding.

4.1.1.3 Diversity array technology (DArT)

Diversity array technology (DArT) is a microarray-based DNA marker technique for genome-wide discovery and genotyping of genetic variation. The proof-of-concept of this technique was initially developed in a species with a simple genome (rice) (Jaccoud et al. 2001). It was advanced to a mature technology in barley, and offers a high multiplexing level while being independent of sequence information.

DArT starts with reducing the complexity of a DNA sample to obtain a 'representation' of that sample by a combination of restriction digestion and adapter ligation, followed by amplification. Two different fragments are amplified from PCR reactions—constant fragments (found in any 'representation' prepared from a DNA sample from an individual belonging to a given species) and variable (polymorphic) fragments (only found in some but not all of the 'representations'). The variable fragments are informative because they reflect sequence variation that determines the fraction of the original DNA sample that is included in the 'representation'. The variable fragments were called DArT markers. To create a library for a given species, a mixture of genomic 'representations' from a pool of individuals covering the genetic diversity of the species is amplified. These fragments are cloned into a vector that is introduced into *E.coli* to form a library. Within the library, each colony contains one of the fragments from the genomic 'representation'. After library creation, a selection of clones from the library was arranged into a plate format (usually 384-well plates). The fragments within the library are amplified and spotted onto glass slides using a microarrayer to form a genotyping array. The genotyping arrays are hybridized with genomic 'representations' of individual DNA samples prepared using the same complexity reduction method. These individual 'representations' are labeled with one fluorescent label, while the vector fragment is labeled with another fluorescent label to act as a reference. Each individual 'representation' will only hybridise to matching fragments on the genotyping array. The slides are then washed and scanned using a scanner to detect fluorescent signals emitted from the hybridised fragments. The presence vs. absence of variable fragments was

recorded as 0 and 1 among individuals of a mapping population.

Diversity array technology (DArT) has been used for genetic map construction in barley (Wenzl et al. 2004) and some other crops (Xia et al. 2005). The validation of the quality and suitability of DArT for mapping and marker-assisted breeding has been demonstrated in the model plant *Arabidopsis thaliana* by evaluating the reproducibility of DArT scores; the consistency of detected genetic differences between parents and their Mendelian segregation in the progeny; the robustness of the genetic linkage map; and the colinearity of the genetic map with the genome sequence map (Wittenberg et al. 2005). Diversity array technology can produce medium-density genome scans comprising several hundred loci at a fraction of the cost of alternative technologies. Due to its non-dependence on sequencing information, high throughput, low cost, and the ability to easily convert DArT markers into other types, this technology could enhance the utility of marker assisted selection in barley breeding programs.

4.1.2 Primary purpose of this chapter

4.1.2.1 Validate the robustness of DArT marker system in a large population

One of the advantages of DArT is that it is a high throughput technology. However, because all the reported studies using DArT were based on sample size of 96 or less (the number of wells in a single plate), it is therefore very important to validate this technique when it is used to genotype a population with a large number of progenies that need to be assayed in multiple batches. Normalization adjusts for a number of technical variations between and within single hybridizations, namely quantity of starting DNA and labeling and detection efficiencies for each sample. A variety of normalization schemes were used during the course of DArT analysis, including dye swap strategies, error checking and quality control, replicates, reference samples, controls, and sensible design of arrays and experiments.

Linkage maps constructed from different sets of genotypes derived from the same cross should closely resemble each other if the methods used to produce the genetic markers and the statistical methods are sufficiently rigorous (i.e., repeatable). Because the same locus order is expected, the comparison of maps from the same individual genotype also provides a way to evaluate the repeatability of genome map construction. The comparison

of two maps constructed from the same individual provides an opportunity to test the reliability of markers and the robustness of the linkage grouping, as well as to screen for segregation distortion. Experimental comparison of maps from the same genotype or different genotypes within the same species has, however, only been rarely reported (Beavis et al. 1991; Plomion et al. 1995).

To investigate the robustness of the DArT technique when it was used to genotype a population with a large number of progenies, genetic linkage maps were generated based on different sub-sets of progenies from a single mapping population screened by a common set of DArT markers. The progenies were genotyped in different batches, and the colinearity of the linkage maps were compared to each other.

4.1.2.2 Investigation of marker segregation distortion

Segregation distortion has been discovered in a large number of taxa and it is increasingly recognized as a potentially powerful evolutionary force (Taylor et al 2003). This was first suggested almost fifty years ago, with the discovery of selfish genetic elements that distort Mendelian segregation to enhance their own transmission (Sandler and Novitski, 1957). It is now believed that they may be important for the evolution of many fundamental aspects of sexual reproduction including the evolution of sex and recombination, the evolution of heteromorphic sex chromosomes, sex ratio evolution, mate choice, and reproductive isolation (Hurst et al. 1996; Werren and Beukeboom 1998; Hurst and Werren 2001; Jaenike 2001).

In plants, segregation distortion was first reported in maize by Mangelsdorf and Jones (1926). Now it has been reported in many other crops including rice (*Oryza sativa* L.; Nakagahra 1972; McCouch et al. 1988; Xu et al. 1997), sorghum (*Sorghum bicolor* L.; Pereira et al. 1994), tomato (*Lycopersicon* sp.; Paterson et al. 1988), alfalfa (*Medicago sativa* L.; Echt et al. 1994), coffee (*Coffea* sp.; Ky et al. 2000), lentil (*Lens* sp.; Vaillancourt and Slinkard 1992, 1993, Eujayl et al. 1998) and barley (*Hordeum vulgare* L.; Graner et al. 1991; Heun et al. 1991; Devaux et al. 1995). It has been suggested that segregation distortion in plants can be due to different biological factors such as chromosome loss, viability problems or lethal genes, genetic isolating mechanisms and genetic load (Bradshaw and Stettler, 1994) although statistical bias, genotyping and scoring errors can not always be ruled out (Plomion et al. 1995) .

DArT, together with RFLP markers, have previously been used for whole genome mapping in barley (Wenzl et al. 2004). The present chapter aims to show that DArT can be used for barley linkage map construction compatibly with SSR and AFLP markers and used to efficiently detect segregation distortion since DArT markers can be well spaced throughout the barley genome.

4.1.2.3 Construction of genetic linkage maps for later use in QTL analysis (chapter 6)

For the purpose of identifying quantitative trait loci controlling barley waterlogging tolerance, a genetic linkage map based on DArT and SSR markers was constructed for a Franklin / Yerong doubled haploid population comprising 182 progenies, and another linkage map based on SSR, AFLP and DArT markers was constructed in a Franklin / TX9425 population.

4.2 Materials and Methods:

4.2.1 Plant materials and DNA extraction

Two doubled haploid (DH) barley populations were used in work reported in this chapter. The first mapping population consisted of 92 doubled haploid lines from a cross between TX9425 and Franklin. TX9425 is a waterlogging tolerant cultivar from China while Franklin is an Australian two-rowed variety with high yield and excellent malting quality. The two parents are phenotypically different in many physiological and agronomic traits (Pang, et al. 2004). The second population consisted of 180 doubled haploid lines from a cross between Yerong and Franklin. Yerong is a six-rowed Australian barley variety with good tolerance to waterlogging stress. The three parents were crossed in Tasmania in 2002. F_1 seeds of Franklin/TX9425 were sown in spring 2003 in a glasshouse in the South Australian Research and Development Institute, with production of dihaploids by Dr Phil Davies. F_1 seeds of Franklin/Yerong were sown in a glasshouse in the Western Australian Department of Agriculture, with dihaploid production by Dr Sue Broughton. The barley plants were grown under a temperature of 20/16 °C (day/night) and daylengths of 16-17 hours in order to produce doubled haploid lines using microspore culture. Fluorescent lights (cool white, Grolux) provided light at approximately 350-400$\mu Em^{-2}s^{-1}$. Barley spikes with microspores at the mid-late uninucleate stage were harvested, outer leaves were removed and spikes were sterilised with 70% ethanol. Spikes were then removed

from the sheath by hand, and the awns gently pulled off. Spikes were subsequently placed into 9 cm petri-dishes and covered with 0.3M mannitol. Dishes were then sealed with Parafilm™ and pretreated by storing at 4°C in the dark for 3-5 days. The isolation, induction, differentiation and regeneration of haploid plants were carried out using Davies' protocol (Davies et al. 1998; Davies 2003). Before transplanting to soil, the haploid plants were rinsed in water to remove media, the roots were kept in 0.1% colchicine solution overnight to double the chromosomes. Genomic DNA was isolated from fresh leaves of the doubled haploid plants using the CTAB extraction method of Saghai-Maroof et al. (1984). DNA concentration was estimated by comparing the fluorescence intensities of ethidium bromide-stained samples to those of DNA standards, on 1% agarose gel. Purified DNA was diluted to a working concentration of 20 ng/μl in steriled water and the stock stored at -20 °C.

4.2.2 DArT protocol

Genomic representations and preparation of the "discovery arrays" and "polymorphism-enriched arrays" were the same as explained by Wenzl et al. (2004). The preparation was carried out in the DArT laboratory in Canberra by myself in collaboration with some members from DArT P/L. Genomic representations of individual barley lines were generated by using the same complexity reduction method as the one used to generate the respective array. Genomic representations were concentrated 10-fold by precipitation with 1 vol of isopropanol, denatured and labelled with 1 μL of 500 μM cy3-labeled random decamers and the exo-Klenow fragment of *E. coli* DNA polymerase I (NEB). Labelled representations (prepared from 92 DH lines) were added to 50 μL of a 50:50:1 mixture of ExpressHyb buffer (Clontech), 10g/l herring sperm DNA, and cy5-labelled polylinker fragment of the plasmid used for library preparation. The DNAs from the DH lines were denatured and hybridised to microarrays overnight at 65°C. Slides were rinsed according to Jaccoud et al (2001) and scanned on an Affymetrix 428 (Santa Lara, CA) adjusting the PMT voltage as required. Spot signal strengths were analysed by DARTSOFT. The software compared the relative intensity values for each individual clone across slides by using a combination of fuzzy C-means clustering at a "fuzziness" level of 1.5 and ANOVA. If two clusters (alleles) could be distinguished and the between cluster variance in relative intensity was at least 80% of the total variance, the clone was said to be polymorphic and scored as 0 or 1. A clone was incorporated into

the 0/1 scoring table of a particular experiment if it was scored with a probability of P > 0.95 in at least 90% of the slides (scoring probabilities were estimated by the clustering algorithm). Individual calls with P < 0.95 were scored as missing. Slides with < 90% of the identified polymorphic markers scored at P > 0.95 were rejected (typically 5%). A quality parameter (variance of the hybridization intensity between allelic states as a percentage of the total variance) was calculated for each marker. Markers with a quality parameter and a call rate both greater than 80% were selected to construct the linkage map.

To test the robustness of the DArT system for linkage map construction, DArT genotyping was performed for 180 doubled haploid lines of the Franklin / Yerong DH population in two separate assays (arrays). The first assay (experiment A) was based on 92 DH lines. The second assay (experiment B) was conducted based on another 88 DH lines. Data from the two assays were standardized and normalized to remove some of the systematic bias.

4.2.3 SSR analysis

142 SSR primers were screened for polymorphism between the two parents and 104 primers showed polymorphisms, a rate of 73.3%. 28 polymorphic primers were selected for genotyping the DH populations, with 4 well-separated primers on each of the seven chromosomes (Ramsay et al. 2000). These SSRs were amplified using fluorescent dUTPs (Molecular Probes, Eugene, Oregon, USA). Amplification reactions were performed in a total volume of 12.5 μL containing 1X Buffer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.2 μM of unlabeled primer, 0.6 μM fluorescent dUTPs, 0.5 U of taq polymerase and 20 ng of template DNA. Amplification conditions were the same as those published at the Genetics supplemental data site at http:/www.genetics.org/cgi/content/full/156/4/1997/DC1. Gel electrophoresis was performed on an automated Gel scanner (Gel-Scan 2000, Corbett Research). Samples were electrophoresed on 18-cm-long 4% polyacrylamide gel containing 7M urea. Allele sizes were calculated by comparison with a 350 (TAMRA) size standard.

4.2.4 AFLP analysis and marker nomenclature

AFLP methodology was performed following Vos et al. (1995). AFLPs were used only

with the Franklin / TX9425 population. Genomic DNA (250 ng) from the two parents and the DH lines was restricted with 2.5 u each of *Eco*RI and *Mse*I in a 20 μl reaction mixture for 2 hours at 37°C. Ligation mixtures of 20 μL containing the *Eco*RI and *Mse*I adaptors, 1 U T4 DNA ligase, 0.4 mM ATP in 10 mM Tris-HCl (pH 7.5), 10 mM magnesium acetate, and 50 mM potassium acetate were added. Ligation mixtures were incubated at 16°C overnight. Ten microlitres of a 5-fold-diluted ligation were amplified for a pre-selective amplification using a pair of primers based on the sequence of the *Eco*RI and *Mse*I adaptors, including one additional selective nucleotide (T) at the 3' end. This was followed by selective amplification reactions which were performed with the *Eco*RI and *Mse*I primers including three additional selective nucleotides at the 3' end (Table 4.5). The *Eco*RI primer was labelled at the 5' end with a fluorophore. The PCR reactions were performed in a 25 μL reaction mixture containing 5 μL of 20-fold-diluted pre-amplified DNA, *Eco*RI and *Mse*I primers, dNTPs, 10X PCR buffer (100 mM Tris-HCl pH 8.3, 15 mM MgCl2, 500 mM KCl), and 0.5 U taq DNA polymerase. Amplification was performed using the following profile: one cycle was performed at 94°C for 30s, 65°C for 30s and 72°C for 60s; followed by 12 cycles in which the annealing temperature was lowered by 0.7°C each cycle; and finally 23 cycles of 94°C for 30s, 56°C for 30s and 72°C for 60s. After the the PCRwas completed, 10 μL of loading buffer (98% v/v formamide, 10 mM EDTA, 0.25% w/v bromophenol blue, 0.25% w/v xylene cyanol) was mixed with 5 μL of amplification products and denatured at 90°C for 3 min. Two μL of each sample was loaded onto 18 cm 6% w/v denaturing polyacrylamide gel with 7.0 M urea and electrophoresed in a 1 % v/v TBE buffer at 1400 V for 1.5 h on the Gel-Scan 2000. Gene Profiler 4.03{3} software was used to extract data and score the traces. All AFLP markers were named using a code for each primer combination (Table 4.5), followed by sequential numbers for scored bands.

4.2.5 Segregation and linkage analysis

To analyse the scored markers, goodness of fit to expected ratios and linkage analysis were performed using JoinMap 3.0 (van Ooijen and Voorrips 2001). Mendelian segregation was tested using Chi-square goodness-of-fit to a 1:1 ratio at a 0.5%, 1% and 5% significance level. For linkage analysis, markers were assigned to tentative linkage groups by using JoinMap's JMGRP module to test LOD thresholds 3.0-7.0 at 0.5 increments. Linkage groups were ultimately assigned on the basis of a LOD threshold of

6.5. Markers within the groups were then analysed for pairwise linkages using JMREC. For this module, REC and LOD thresholds of 0.499 and 0.01, respectively, were used according to the JoinMap's recommendations. The linkage groups were then ordered with JMMAP using the following parameters: a 0.1 JMMAP LOD threshold, a 0.45 REC threshold, 2 jump threshold, 7 triplet threshold, 1 ripple value, and Kosambi's mapping function. A second round of analysis was undertaken, where the markers with segregation distortion were removed, to construct a framework. Comparison of marker order between maps was done on a chromosome scale.

4.3 Results

4.3.1 Validation of the robustness of the DArT marker system in Franklin x Yerong population

4.3.1.1 The individual DArT linkage maps based on the two subsets of progenies from Franklin / Yerong population

The linkage maps based on the two subsets of progenies (experiment A and B) were separately generated. The linkage analysis in experiment A shows that the DArT markers were grouped into 13 linkage groups at LOD 6.5, while 14 groups were defined at the same LOD level in experiment B (Table 4.1). All the groups in the two sub- populations were assigned to the seven barley chromosomes by comparing the genetic linkage maps of these sub populations with a new barley integrated map based on DArT markers (in Chapter 5). The results indicated that although there is one more group in population B than in population A, all the 496 markers assayed over the sets of progenies were assigned exactly to the same seven different barley chromosomes in each of the two experiments, with no marker showing any difference in its assignment to barley chromosomes (Fig.4.1). In experiment A, chromosome 1H, 2H, 3H and 5H all split into 2 linkage groups, chromosome 4H split into three linkage groups (Fig. 4.1; Table 4.1). Whereas in experiment B (Fig 1; Table 4.1), chromosome 2H, 3H and 4H split into two linkage groups, and chromosome 1H and 5H split into three groups (Table 4.1; Table 4.2). The genetic distance of the linkage map of population A is 932.3 cM, while that of population B is 940.1 cM (Table 4.2).

Table 4.1 Comparison of the number of DArT markers assigned to each chromosome between the two experiments (sets of progenies) (Franklin / Yerong cross) analyzed separately. The number of groups (if a chromosome was separated into more than one group) and the number of markers within each group are indicated.

	Chromosomes							
Experiment	1H	2H	3H	4H	5H	6H	7H	Total
A	54, 26	48, 40	39, 31	17, 6, 4	43, 15	72	101	
total	80	88	70	27	58	72	101	496
B	43, 26, 11	48, 40	39, 31	21, 6	38, 15, 5	72	101	
total	80	88	70	27	58	72	101	496

Table 4.2 Comparison of chromosomal lengths (cM) and the total genome length between the two experiments (Franklin / Yerong cross) analysed separately. The number of groups (if a chromosome was separated into more than one group) and the lengths of each group were indicated.

	Chromosomes							
Experiment	1H	2H	3H	4H	5H	6H	7H	Total
A	56.9, 57.9	68.9, 82.8	100.5, 40.8	22.3, 29.2, 21.6	116.7, 30.2	150.4	154.2	
total	114.8	151.7	141.3	73.1	146.9	150.4	154.2	932.3
B	39.2, 56.0, 4.4	49.5, 88.1	92.3, 52.1	69.6, 35.3	90.2, 21.2, 22.6	134.7	185	
total	99.6	137.6	144.4	104.9	134	134.7	185	940.1

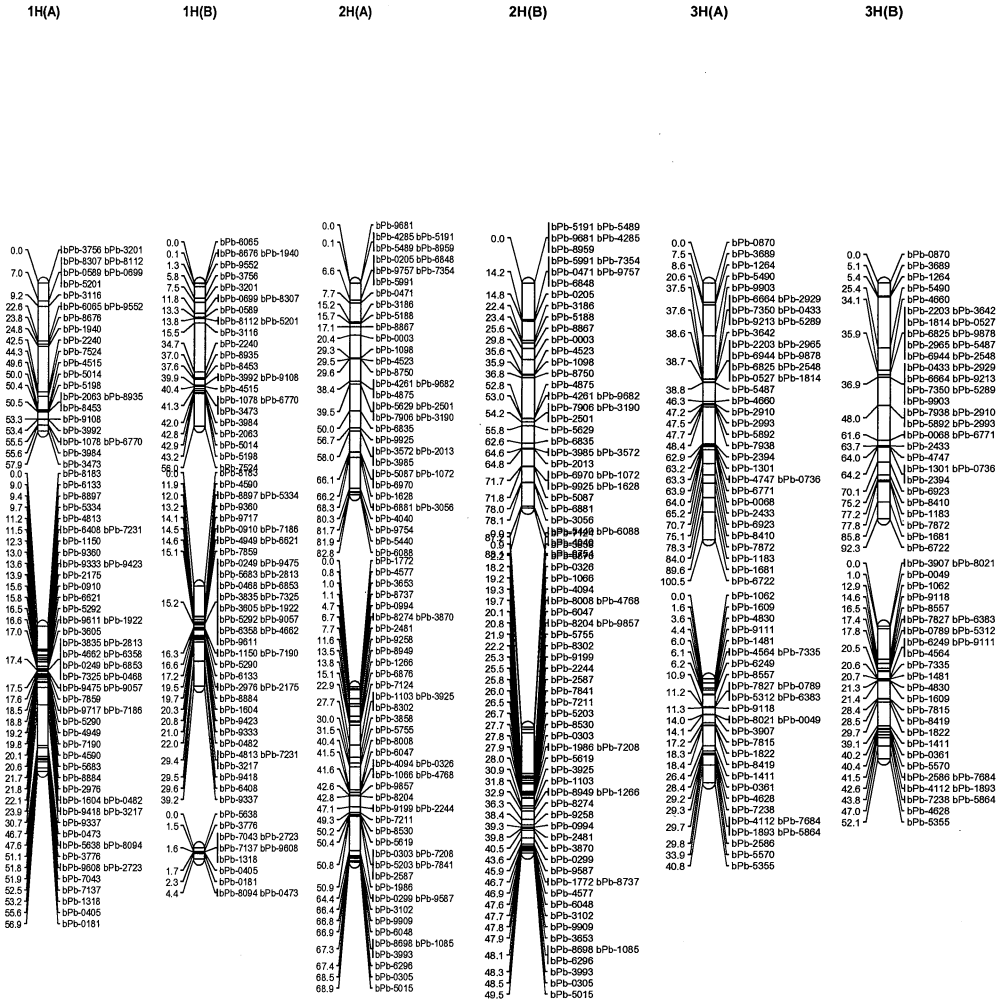
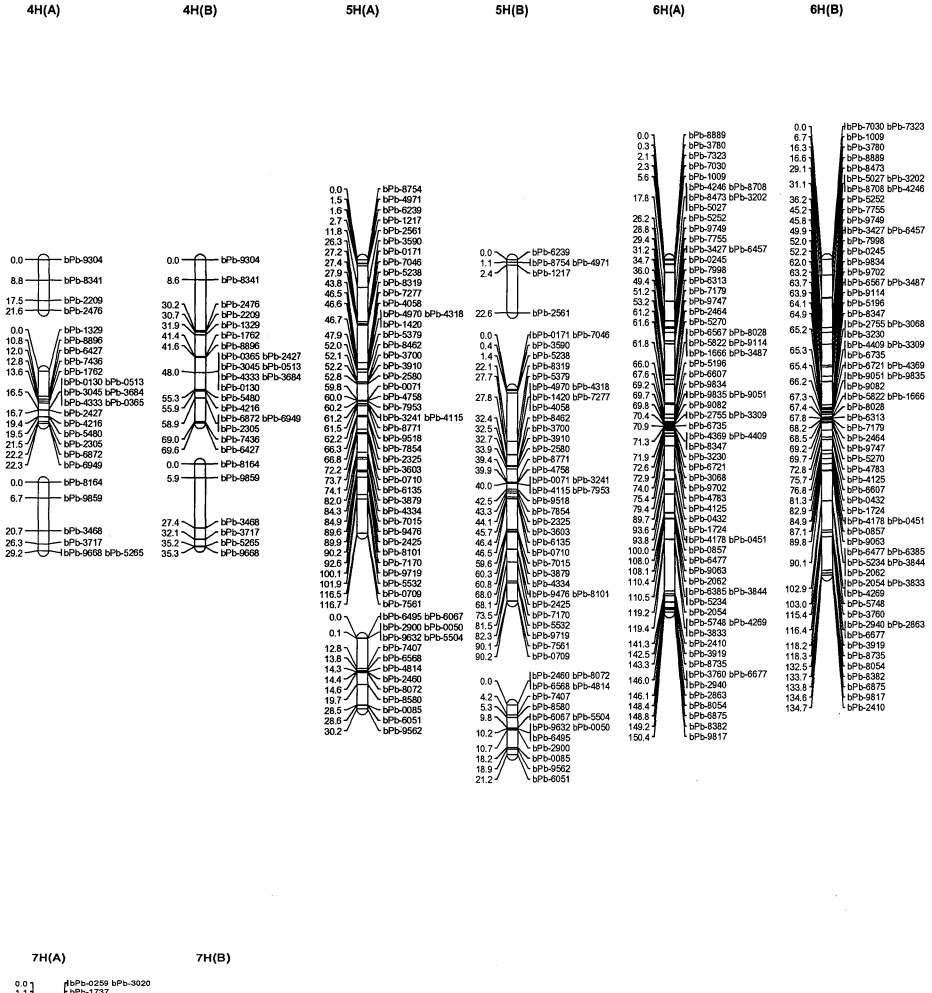


Fig. 4.1 Colinearity of the genetic linkage maps based on two different sets of progenies (experiment A and B) from Franklin / Yerong population using only DArT markers. The linkage groups associated with a barley chromosome were placed one on top of another and placed side by side for the two experiments based on the linkage knowledge from the overall analysis for this population. Distance between markers is indicated on left side of each linkage group in cM. Enlarged version of this figure is in Appendix 1.



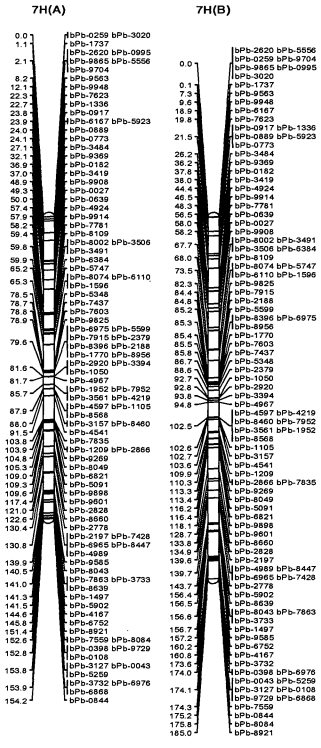


Fig. 4.1 continued

4.3.1.2 Co-linearity between the two maps

As already explained, the DArT markers were assigned to exactly the same chromosome in the two replicated maps. Comparison of marker order was done between the two maps one chromosome at a time. The result shows that marker order is highly similar with some minor rearrangements of marker orders at small map intervals of less than 5 cM, but no large rearrangement between the two maps was found except one on chromosome 2H and one on 3H (Fig. 4.1). To ascertain whether this observation was representative for the data, a statistical technique called 'bootstrapping' was used to resample the data from the experiment and to generate 'new' maps to compare with the original ones. Two new different sets of individuals were sampled with replacement from the pool of 182 individuals. Two new maps were constructed and were compared with the original maps. The number of differences in locus order was very similar to the original maps. Thus, observation of order differences based on the comparison of two maps appeared to be representative of the variation in locus order that can be expected given the effect of sample size on calculation of distance between loci. No large rearrangements between the two maps indicated that there is little genotyping error or data variation in the DArT marker system when it assays a large number of samples over different assays.

4.3.2 Construction of an overall linkage map of Franklin / Yerong population based on DArT and SSR markers

4.3.2.1 Segregation distortion analysis

Marker segregations were tested for deviation from the expected Mendelian segregation (1:1) by chi-squared analysis. Among the 518 markers, 32.8% showed segregation distortion at the 0.05 level of significance, 20.8% at the 0.01 level and 16.2% at the 0.005 levels. The segregated markers were not distributed randomly, for example, at the 0.01 level of significance, 30.3% and 34.3% of the total markers with distorted segregation were mapped to chromosome 5H and 6H respectively (Table 4.3). At the 0.005 level, 35.1% and 40.3% of the distorted segregation markers were mapped to chromosomes 5H and 6H, respectively, with a total of 75.4%. Because about 75.4% of segregation distorted markers were mapped to chromosomes 5H and 6H at $p \leq 0.005$), segregation distortion in this cross is most likely to be caused by genetic factors. Chromosomal scale segregation distortion analysis showed that 68.4% and 57.3 % of the markers on chromosomes 5H

and 6H respectively showed segregation distortion at p ≤0.05 (Table 4.3; Fig.4.2).

Table 4.3 Chromosome-scale segregation distortion test calculated as the proportion of segregation distorted markers to total markers on each chromosome and as the proportion of distorted markers on each chromosome to the total distorted markers at p ≤0.05, 0.01 and 0.005 (Franklin / Yerong cross).

Linkage group	Percentage of distorted markers to total markers on chromosome basis at p ≤0.05, 0.01 and 0.005			Percentage of distorted markers on each chromosome to distorted markers in the whole genome		
	p ≤0.05	p ≤0.01	p ≤0.005	p ≤0.05	p ≤0.01	p ≤0.005
1H1	27.78	3.7	1.85	9.62	2.02	1.3
1H2	19.16	0	0	3.21	0	0
2H1	17	8.5	2.12	5.13	4.04	1.3
2H2	32.43	24.32	10.81	7.69	9.09	5.19
3H	20	10.77	3.08	8.33	7.07	2.86
4H	14.29	3.57	0	2.56	1.01	0
5H	68.42	52.63	47.37	25	30.3	35.1
6H	57.35	50	45.59	25	34.3	40.3
7H	22.34	12.77	11.7	13.5	12.1	14.3

4.3.2.2 Genome coverage and marker distribution

For the purpose of identifying the quantitative trait loci (QTLs) controlling barley waterlogging tolerance in a later chapter (Chapter 6), a genetic linkage map, based on 182 progenies from the Franklin / Yerong population, was constructed based on DArT and SSR markers. A total of 518 markers (496 DArT markers and 22 SSR markers) were used in the linkage analysis.

To construct the linkage map different LOD thresholds (from LOD 3 to LOD 7) were first tested to group the markers. A LOD threshold of 5.5 resulted in the optimum number of markers in linkage groups in which linkage order and distances were maintained. JoinMap3.0 recommends using a JMMAP LOD in the range of 0.01 to 1.00 for ordering markers and a REC value between 0.45 and 0.49 to create pairwise distances between

markers. A stepwise increase in the JMMAP LOD threshold from 0.01 to 0.2 in the ordering phase of analysis made little difference in map order and distances, confirming integrity of marker data and group results. Analyses were conducted with a JMMAP LOD threshold of 0.1. REC thresholds were tested from 0.45 to 0.49 with stepwise increments of 0.01. The REC threshold made very little difference in distances for each group, and the total distance for the whole genome was stable. A REC threshold value of 0.45 was finally used. In order to obtain a rigorous marker order, the framework map was first optimised using only non-distorted markers. Distorted markers were then added in a second step and integrated into the map framework. In most cases, the introduction of distorted markers did not affect the statistical confidence of marker order, or just changed the order of markers within very small regions with a high density of markers.

Nine linkage groups were defined at a LOD threshold of 5.5. Markers were mapped to 476 positions covering a total map distance of 1084.5 cM (Table 4.4) in nine linkage groups. Chromosomes 1H and 2H were split into two linkage groups. Linkage groups were assigned to the seven chromosomes by using SSR markers as anchor markers. The average distance between markers is 2.28 cM, but markers were not distributed evenly across linkage groups. Linkage group 4H had the lowest density of markers with an average interval of 5.0 cM, compared to the highest density of 1.3 cM per interval on linkage group 1H1 (Table 4.4). Markers on each linkage group were distributed fairly randomly, except for a few clusters on linkage groups 1H, 2H and 7H. However, there were several large gaps with distances over 20 cM on chromosomes 1H, 3H, 4H and 5H. Other large gaps, presumably, resulted in the split of chromosome 1H and 2H into two linkage groups (Fig.4.2).

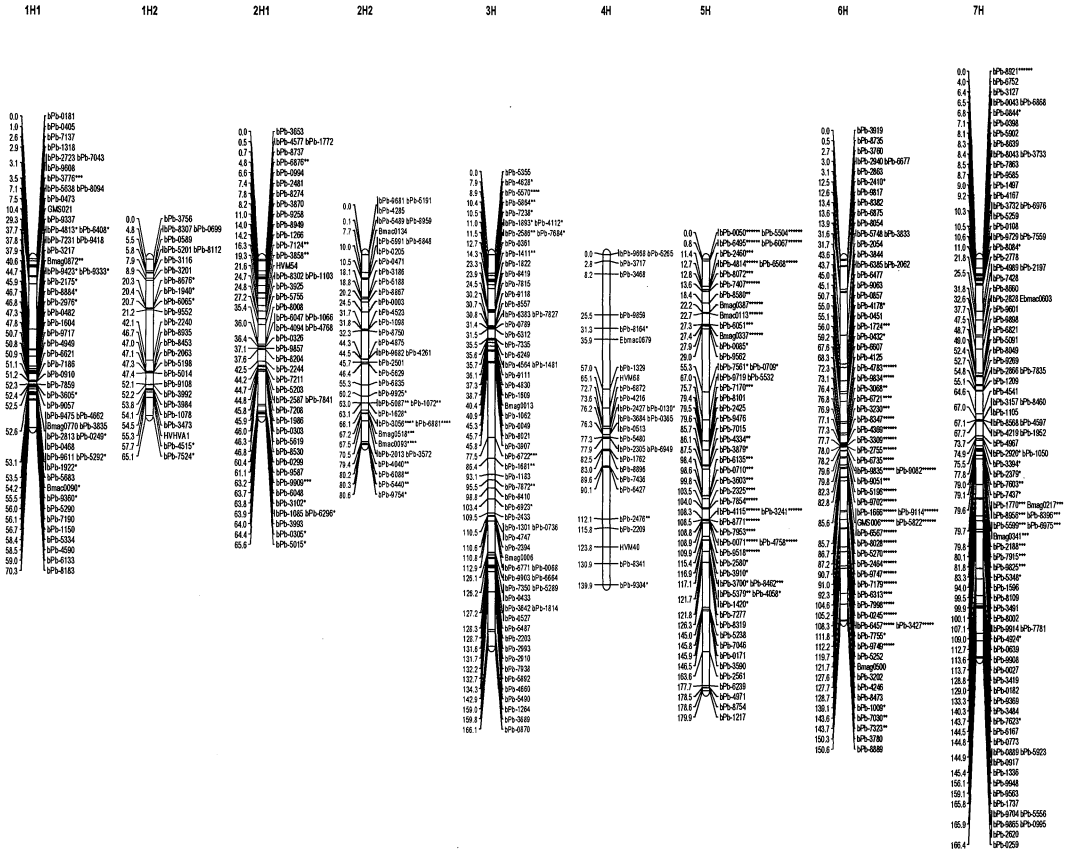


Fig. 4.2 The overall linkage map based on 180 DH progenies from Franklin / Yerong population using DArT and microsatellite markers. Markers with segregation distortion were indicated with stars (*), with P < 0.05 = *; P < 0.01 = **; P < 0.005 = ***. The linkage groups are named and organized by chromosomes, e.g. chromosome 1H had two linkage groups 1H1 and 1H2. DArT markers are encoded with the letter bPb followed by a number, the other markers are all microsatellites. Enlarged version of this figure is in Appendix 2.

Table 4.4 Linkage group size, number of markers, and average marker interval per linkage group in Franklin / Yerong cross

Linkage group	Size (cM)	No. of markers	Average marker interval (cM)
1H1	70.3	54	1.3
1H2	65.1	26	2.5
2H1	65.6	47	1.4
2H2	80.6	37	2.18
3H	166.1	65	2.56
4H	139.9	28	5
5H	179.9	57	3.16
6H	150.6	68	2.21
7H	166.4	94	1.77
Whole genome	1084.5	476	2.27

4.3.3 Construction of genetic linkage maps of Franklin / TX9425 population based on SSR, AFLP and DArT markers

4.3.3.1 Map construction and segregation analysis

424 DArT markers were obtained in this population. The amount of missing data for any one marker varied between 0 and 20%, and averaged 5.4%. Twelve of these markers had very strong segregation distortion (85:7 or worse) and were removed from the data set. The remaining 412 markers were scored with an overall call rate of 95%. Seventeen samples were assayed in duplicate and in these samples only 37 scoring errors were obtained in more than 6800 comparisons, an accuracy rate of 99.5%. A quality parameter Q (variance of the hybridization intensity between allelic states as a percentage of the total variance) was calculated for each marker. The result shows that markers with Q

above 75 were often very consistent. Only markers with a quality parameter and a call rate both greater than 80% were selected to construct the linkage map.Using 19 primer combinations, a total of 176 polymorphic AFLP markers (Table 4.5) were obtained. All these AFLP markers were graded into three categories (category 1, clearly scorable; category 2, scorable; category 3, difficult to score) according to the quality of the polymorphic bands. Only 81 markers from category 1 were used for mapping. One SSR marker was removed (HVM68) because it suffered from very strong segregation distortion (89:3), this left 27 SSR markers for further analysis.

Marker segregations were tested for deviation from the expected Mendelian segregation (1:1) by chi-squared analysis. Among the 520 markers, 40.9% showed segregation distortion at the 0.05 level of significance, 27.2% at the 0.01 level and 20.2% at the 0.005 levels. More alleles from the under-represented class originated from TX9425 than from Franklin (91 for Franklin *versus* 133 for TX9425; $c^2_{1df} = 7.9$, $P < 0.0005$). A larger proportion of AFLP (50.6%) and SSR (48.0%) than DArT (38.6%) markers showed segregation distortion at the 0.05 threshold level. DArT markers from the under-represented class were as often from the 0 (44%) as from the 1 (55%) class ($c^2_{1df} = 1.0$, NS). The distorted markers were not distributed evenly through the whole genome. For example, about 41% of the total number of segregation distorted markers at $P = 0.005$ were mapped to chromosome 3H (Table 4.6). LOD thresholds (from LOD 3 to LOD 7) were tested to group the markers, and a LOD threshold of 3.5 resulted in the optimum number of markers in linkage groups in which linkage order and distances were maintained. Marker order analyses were conducted with a JMMAP LOD threshold of 0.1 and a REC threshold value of 0.45. In order to obtain a rigorous marker order, a framework map was constructed using only non-distorted markers. Distorted markers were then added in a second step and integrated into the map framework. In most cases, the introduction of distorted markers did not affect the statistical confidence of marker order, or just changed the order of markers within very small regions with a high density of markers. But markers with segregation distortion were not dispersed randomly between the eight linkage groups (Fig.4.3; Fig 4.4; Table 4.6; $c^2_{7df} = 83.3$, $P < 0.001$). It is also interesting that the distorted markers on each chromosome were clustered into regions where overall marker densities were very high (Fig.4.3; Fig 4.4).

Table 4.5 AFLP primer pair combinations and number of polymorphic and recorded markers for each primer combination. Recorded markers were those that were clearly scorable (category 1).

Primer combination	Selective nucleotides		polymorphic products	recorded markers	% of recordable markers
	*Eco*RI	*Mse*I			
1	ATT	CGC	16	9	56.3
2	AAA	CCG	15	11	73.3
3	ATG	CTG	9	6	66.7
4	AAC	CTC	10	7	70
5	ACT	CGG	9	6	66.7
6	ACA	CCC	11	8	72.7
7	ATG	CGC	14	11	78.6
8	AAC	CGC	17	13	76.5
9	ATG	CCG	11	7	63.6
10	AAC	CCG	13	10	76.9
11	ACT	CTG	11	6	54.5
12	AAG	CTG	15	11	73.3
13	ATG	CTC	14	10	71.4
14	ACA	CTC	13	10	76.9
15	AAG	CGG	16	9	56.3
16	ATG	CAG	17	13	76.5
17	ACA	CAG	18	11	61.1
18	ATT	CGA	17	10	58.8
19	ACT	CGC	14	8	57.1
Total			244	176	
Mean					72.1

Table 4.6 Chromosome-scale segregation distortion test calculated as the proportion of segregation distorted markers to total markers on each chromosome and as the proportion of distorted markers on each chromosome to the total distorted markers at p ≤0.05, 0.01 and 0.005 (Franklin / TX9425 cross).

Linkage groups	Percentage of distorted markers mapped to each chromosome to total number of distorted markers at p ≤ 0.05, 0.01 and 0.005.			Percentage of distorted markers to total number of markers on each chromosome at p ≤0.05, 0.01 and 0.005.		
	p ≤0.05	p ≤0.01	p ≤0.005	p ≤0.05	p ≤0.01	p ≤0.005
1H	11.5	12.4	10.8	39.1	26.1	19.6
2H	7.1	7.2	7.2	12	7.6	6.5
3H	37.8	47.4	41.4	74.7	58.2	58.2
4H	6.4	6.2	2.4	58.8	35.3	11.8
5H	14.1	11.3	9.6	31.4	15.7	11.4
6H1	6.4	3.1	3.6	27.8	8.3	8.3
6H2	5.1	2.1	1.2	44.4	11.1	5.6
7H	11.5	10.3	9.6	28.6	14.3	11.1

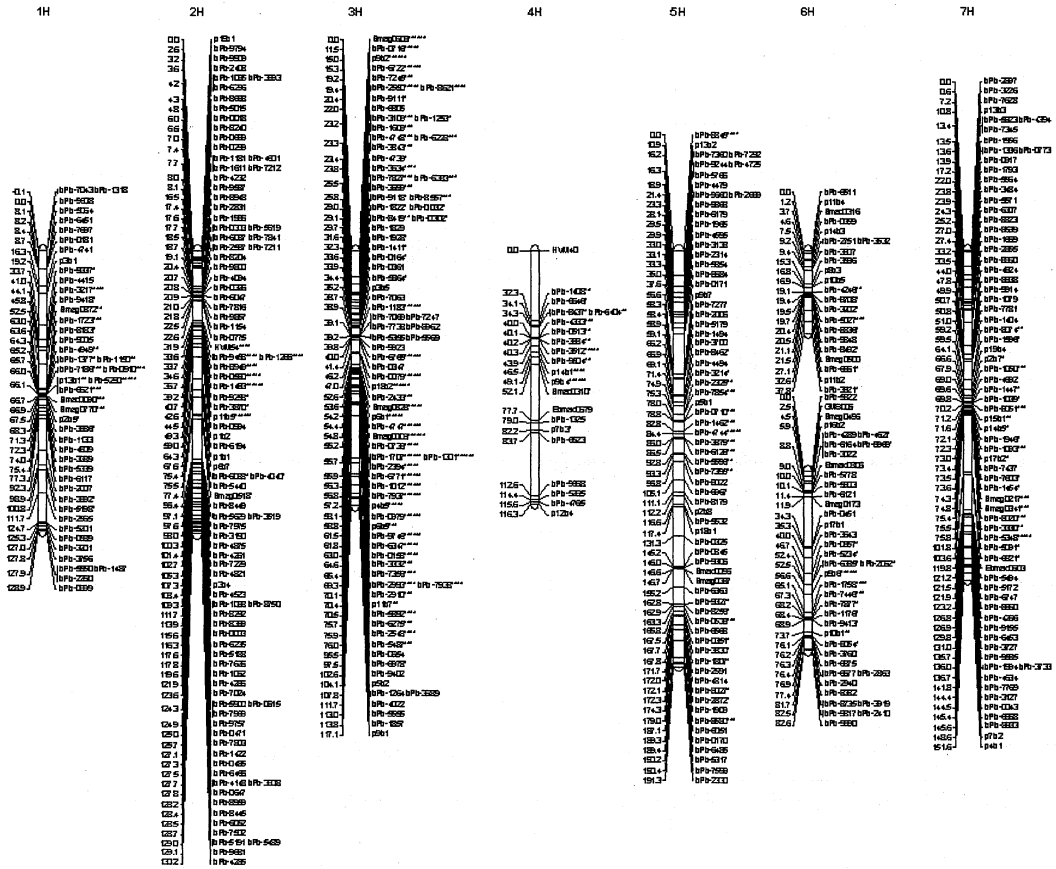


Figure 4.3 Barley genetic linkage map of Franklin / TX9425 population based on DArT, AFLP and SSR markers. Markers with segregation distortion were indicated with stars (*), with $P < 0.05$ = *; $P < 0.01$ = **; $P < 0.005$ = ***. The linkage groups are named and organized by chromosomes, e.g. chromosome 1H had two linkage groups 1H1 and 1H2. DArT markers are encoded with the letter bPb followed by a number; AFLP markers were named using a code for each primer combination followed by sequential numbers for scored bands; other markers are microsatellites. Enlarged version of this Figure is in appendix 3.

4.3.3.2 Genome coverage and marker distribution

The final genetic map comprised of 450 markers organized into 8 linkage groups spanning a genetic distance of 956 cM (Fig.4.3). Seventy markers were dropped from the analysis because they mapped to identical positions as other markers. Linkage groups were named using the position of known SSRs (Ramsay et al. 2000). The average

distance between two markers across the whole map was 2.12 cM (Table 4.7). Markers were not distributed evenly across linkage groups; linkage group 4H had the lowest density of markers with an average interval of 5.52 cM, compared to the highest density of one marker every 1.34 cM on chromosome 2H. Markers on each linkage group were distributed fairly randomly, except for a few clusters on linkage groups 1H, 2H and 7H. However, there were several large gaps with distance over 20 cM on linkage groups 4H, 6H1 and 7H. Another large gap, presumably, resulted in the split of chromosome 6 into two linkage groups (6H1 and 6H2).

Table 4.7 Linkage group size, number of markers, and average marker interval per linkage group in the population of Franklin / TX9425.

Linkage groups	Size (cM)	No. of markers	Average marker interval (cM)
2H	130	97	1.34
3H	117	84	1.39
7H	152	71	2.14
5H	191	69	2.77
1H	129	46	2.8
6H1	83	40	2.08
6H2	38	21	1.81
4H	116	21	5.52
Total	956	450	
Mean			2.12

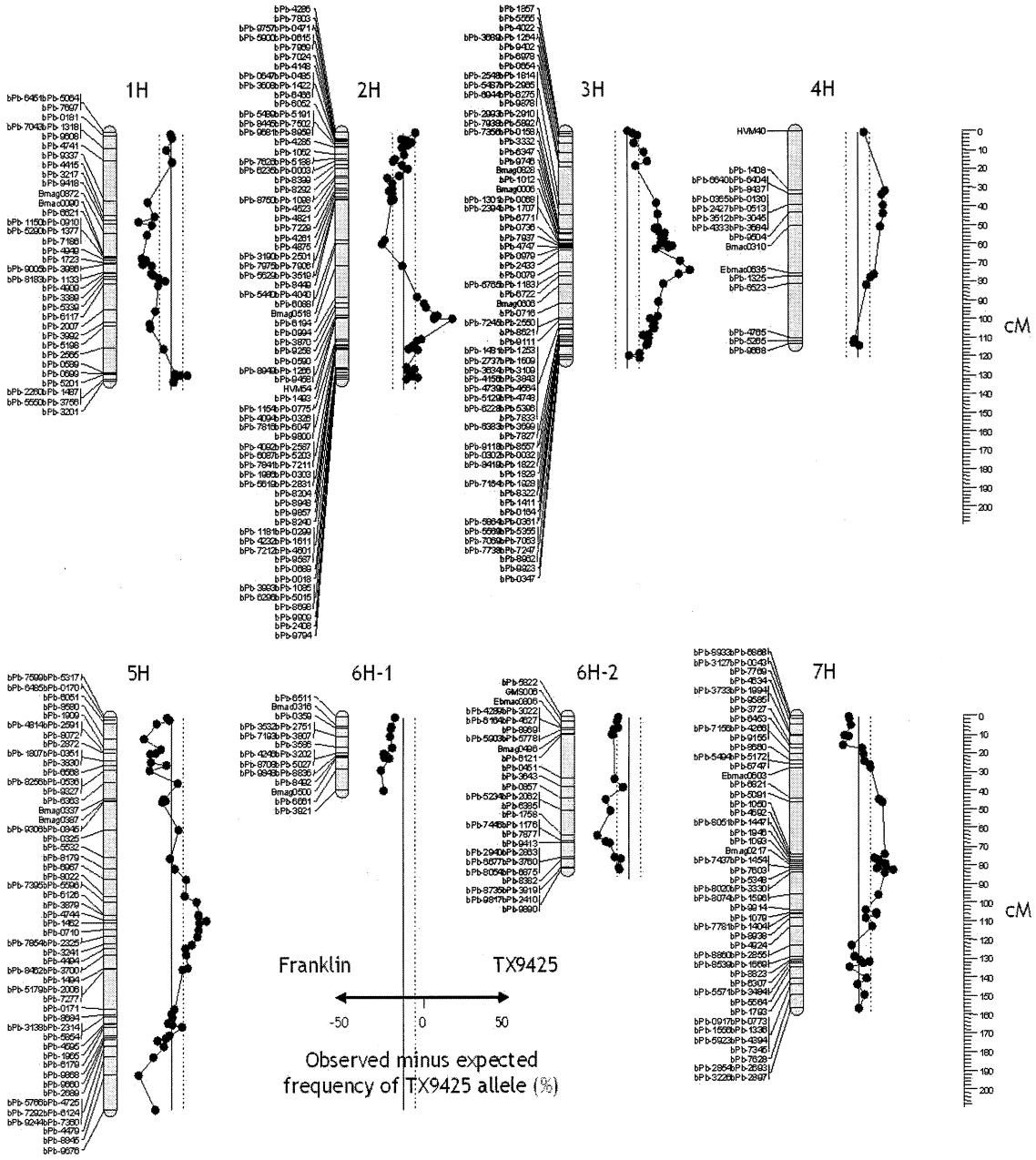


Figure 4.4 Barley genetic linkage map of Franklin/TX9425 based on DArT, AFLP and SSR markers. Adjacent XY (scatter) graphs depict segregation distortion for each marker. The Y axis is distance in centimorgans and X axis is the difference between the observed minus expected frequency of TX9425 allele (%) assuming 1:1 segregation. The solid vertical line indicates O-E of zero, with the adjacent dotted lines indicating plus or minus 5% distortion

4.4 Discussion

4.4.1 Diversity array technology (DArT)

DArT markers for a given species are discovered by screening a library of several thousand fragments from a genomic representation prepared from a pool of DNA samples that encompass the diversity of the species. The microarray platform makes the discovery process very efficient because all markers on a particular DArT array are scored simultaneously (Jaccoud et al. 2001). It can be used to create medium-density genetic maps for plants with complex genomes taking only several days (Wenzl et al. 2004). As was indicated in the experiment reported here, DArT was very robust when it was used for genotyping a large number of individuals assayed in different batches. The nature of high-throughput and independence from sequence information enables routine use of DArT in plant breeding programs, such as exhaustive fingerprinting of germplasm, quantitative trait locus identification, simultaneous marker assisted selection of several loci, and accelerated introgression of interesting genome regions. High density maps for map-based cloning and chromosome-landing approaches could be rapidly built by pyramiding data from a number of independent arrays (Wenzl et al. 2004).

4.4.2 Map characteristics and genome coverage

To my knowledge no previous genetic map for barley based on DArT, AFLP and SSR markers has been reported. For the purpose of identification of quantitative trait loci controlling waterlogging tolerance in barley, two genetic linkage maps were constructed in this chapter. The genetic map of Franklin/TX9425 comprised of 450 markers organized into 8 linkage groups, instead of the expected 7 groups, because chromosome 6H was split into two groups. The linkage map of Franklin/Yerong is based on 496 DArT and 22 SSR markers, they were assigned to 9 linkage groups with chromosomes 1H and 2 H split into two linkage groups. Linkage maps covered 956 cM in the Franklin/TX9425 population and 1084.5 cM in Franklin/Yerong population. The total length of these two maps were similar to some AFLP linkage maps with total map lengths of 900-1100 cM (Powell et al. 1997; Yin et al. 1999), but was shorter than other barley linkage maps with total lengths of 1100-1300 cM (Kleinhofs et al. 1993; Ramsay et al 2000). It has been reported that maps constructed using Joinmap are often shorter than those constructed

using MAPMAKER (Sewell et al.1999 in loblolly pine and Qi et al.1996 in barley). The size difference between these maps and other published maps may also result from the lower genome homology between two parents of these crosses, reducing recombination frequency in the F1 and map size (Bonierbale et al. 1988; Gebhardt et al. 1991; Vaillancourt and Slinkard 1993; Paillard et al. 1996). The clustering of markers observed here may be due to either centromere and / or telomere suppression of recombination (Tanksley et al. 1992) or to a tendency of some molecular markers to map in clusters (Qi et al. 1998; Vuylstecke et al. 1999).

4.4.3 Segregation distortion

Marker distortion has been reported in numerous cases in plants. The percentage of loci showing segregation distortion has been highly variable between different studies: 69% in *Cryptomeria japonica* (Nikaido et al. 1999), 37% in *Citrus* (Luro et al. 1994), 36% in *Oryza* (Xu et al. 1997; Virk et al. 1998), 33% in *Prunus* (Foolad et al. 1995), 23% in *Helianthus* (Quillet et al. 1995), 8.4% in *Lens* sp. (Eujayl et al. 1998) and only 1.4% in *Hevea* spp. (Lespinasse et al. 2000). Distorted segregation has been repeatedly observed in anther culture-derived or microspore-derived barley (Thompson et al. 1991; Graner et al. 1991; Heun et al. 1991; Zivy et al.1992). Segregation distortion of 41% of the markers in Franklin / TX9425 and 32.8% in Franklin/Yerong reported here was comparable with segregation distortion levels found for other androgenetic-derived mapping populations in barley (44%, Graner et al. 1991; 44.1%, Manninen 2000), although one of the crosses reported here involved two geographically and presumably distantly related varieties of barley, TX9425 from China and Franklin from Australia. The other cross was made between a six-rowed (Yerong) and a two-rowed (Franklin) barley variety with significant differences in stress tolerance.

Skewed segregation ratios can be detected with almost any kind of genetic marker, including morphological, isozymes and DNA markers (Foltz 1986; Zamir and Tadmor 1986; Vaillancourt and Slinkard 1993; Abe and Tsuda 1987; Wendel et al. 1987; Bundock et al. 1990; Konishi et al. 1990). Many types of molecular markers, such as RFLP (Graner et al. 1991; Heun et al. 1991; Devaux et al. 1995), RAPD (Manninen, 2000), AFLP (Qi et al.1996; Becker and Heun, 1995), SSR (Sayed et al. 2002) but so far no DArT markers have been used for detection of segregation distortion in barley.

Several reasons for distortion of segregation ratios in plants have been put forward, including such factors as chromosome loss (Kasha et al. 1970), genetic isolation mechanisms (Zamir et al. 1986), and the presence of viability genes (Hendrick et al. 1990; Beavis et al. 1991; Liedl et al. 1993; Bradshaw et al. 1994). Nonbiological factors such as scoring errors (Devey et al. 1994; Xu et al. 1997; Nikaido et al. 1999) and sampling errors (Plomion et al. 1995; Echt et al. 1997) can also lead to distortion in segregation ratios. There is no evidence that the segregation distortion in this cross was caused by genotyping and scoring errors using the DArT markers, since they suffered a rate of distortion that was lower than that of the SSR and AFLP markers. A total of 64.6% of segregation distorted markers were mapped to chromosomes 5H and 6H in population Franklin / Yerong, and about 47.4% of segregation distorted markers in Franklin / TX9425 cross were mapped to chromosome 3H at $p = 0.01$ indicating that segregation distortion in these two crosses is most likely to be caused by different genetic factors.

The genetic control of distorted segregation has previously been studied using morphological and isozyme markers. Distortion has usually been observed to be in favour of the allele of the parent showing superior performance in *in vitro* culture (Foisset and Delourme 1996). In the present study, I found that much stronger distortions of single locus segregations were observed in the anther/microspore culture-derived barley DH populations (Franklin/TX9425 and Franklin/Yerong) than in the *Hordeum bulbosum*-derived populations (Clipper/Sahara and Steptoe/Morex, for the details of the latter two populations see Chapter 5 of this thesis). This conclusion was in agreement with the previous reported studies (Devaux, et al. 1995).

Many genes are expressed postmeiosis during microspore and pollen development in angiosperms (Mascarenhas 1992), thus if any of these genes are variable there may be selection. Alternatively, segregation distortion could have arisen from hybrid sterility genes that caused the abortion of specific gamete or zygote genotypes. These partial lethal factors may make an androgenetic plant population show an increased rate of segregation distortion. Two regions on chromosomes 1H and 5H in anther-derived DHs from the F_1 between Steptoe and Morex showed strong distortion (Devaux et al. 1995). Similarly, distorted loci were reported in DH populations from other crosses on chromosomes 1H (Kintzios et al. 1994), 5H (Steffenson et al. 1995), 4H and 6H (Thompson et al. 1991), 2H and 7H (Logue et al. 1995) and 3H (Graner et al.1991;

Devaux et al. 1995). The distorted regions were found on almost all chromosomes in the Franklin / TX9425 population with a significant portion (47.4%) of the distorted markers mapped to chromosome 3H ($p \leq 0.01$, whereas 75.4% ($p \leq 0.005$) of distorted markers were mapped to chromosomes 5H and 6H in Franklin/Yerong, indicating the complexity of determining the factors contributing to segregation distortion.

To understand the underlying mechanisms that cause segregation distortion, it would be useful to map the partial lethal-factor loci on different linkage maps and compare them in different crosses, or to develop near isogenic lines containing individual segregation distortion loci, so that the effect of these factors could be evaluated systematically in different genetic backgrounds and environments

Chapter 5 The construction of a new barley consensus map and the comparison of chromosomal regions associated with segregation distortion in barley

5.1 Introduction

Barley is an excellent system for genome mapping and map-based analysis because it is diploid. The species has seven cytologically distinct chromosomes containing approximately 5.3 x 10^9 base pairs (bp) of DNA (Bennett and Smith 1976). Although barley is an autogamous species, there is sufficient DNA-level diversity for efficient linkage map construction in populations derived from crosses between related genotypes using RFLP, RAPD, SSR and AFLP markers (Graner et al. 1991; Kleinhofs et al. 1993; Kasha et al. 1995; Becker et al. 1995; Hayes et al. 1997; Powell et al. 1996; Russell et al. 1997; Gamsay et al. 2000). These linkage maps are useful from the standpoint of understanding genome organization, establishing synteny, as a platform for map-based cloning, and for QTL detection. However, each of these individual genetic maps was constructed by different research groups, for their own purposes. The information contained within these individual maps could be further enhanced if these maps were to be synthesized into a single consensus map to represent this species.

Integrating independent maps presents a challenge to geneticists because of the inherent inconsistencies and ambiguities embodied by each map. No standard procedure for map integration has been generally agreed upon. There are four main approaches to integration that have been described in the literature. The simplest approach is to visually align different maps on the basis of common markers. This visual approach was used to create a 'consensus map' of the homeologous groups of wheat (Nelson et al. 1995a, 1995b, 1995c; Van Deynze et al. 1995; Marino et al. 1996). Kianian and Quiros (1992) also used this approach to create a 'composite map' for *Brassica oleracea*. A second approach was used by Beavis and Grant (1992), who generated an integrated map for maize using MAPMAKER after pooling all of the marker data from different mapping populations with similar size and structure. The third approach is to use software such as Joinmap,

which weights the distances between markers by the structure and size of each population. This technique was used to produce a consensus map for loblolly pine (Sewell et al. 1999) and sugi (*Cryptomerica japonica*) (Tani et al. 2003) on the basis of two pedigrees. The fourth approach was described by Yap et al. (2003). This approach integrates maps by modelling maps as graphs. A specific map is designated as the primary or standard map, then additional maps are successively projected onto the standard. It allows for comparisons purely on the basis of marker order and does not require access to the raw mapping data or information about distances between markers. It can also be used to integrate maps of different types, such as genetic, physical, or sequence based maps (Yap et al. 2003).

With the development of these approaches for map integration, consensus maps have been constructed for a number of plant species (e.g., *Arabidopsis thaliana*, Hauge et al. 1993; *Brassica oleracea*, Kianian and Quiros 1992; *Helianthus annuus*, Gentzbittel et al. 1995). Several barley consensus maps have also been built with gel-based markers, such as restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP) (Langridge et al. 1995; Qi et al. 1996; Karakousis et al. 2003; Diab 2006; Varshney et al. 2006). These maps have provided an important framework for producing and exchanging genetic information among members of the barley scientific community.

Mapping with multiple populations provides several advantages over mapping based on a single population. In particular, a larger number of loci can be placed onto a single map. This is especially important when attempting to map specific genes of interest (e.g., morphological markers or candidate genes for trait mapping) that are unlikely to segregate within a single mapping population. This also provides for better genomic coverage. These multi-population mapping studies have provided evidence for chromosome rearrangements (Beavis and Grant 1991; Kianian and quiros 1992) and gene duplication (Kianian and quiros 1992; Gentzbittel et al. 1995), have assisted in the assignment of linkage groups to chromosomes (Beavis and Grant 1991), and have provided the basis for comparative studies among related species and subspecies (Kianian and Quiros 1992; Hauge et al. 1993; Gentzbittel et al. 1995).

Numerous types of DNA-based genetic marker have been developed over the past two

decades. However, most of these genotyping methods are constrained by their dependence on gel electrophoresis, resulting in low throughput. Methods based on electrophoresis also suffer from difficulties in precisely correlating bands on gel with allelic variants. To overcome these restrictions, a high-throughput microarray-based DNA marker technique - Diversity Array Technology (DArT) has been developed (Jaccoud et al. 2001). It has been used for genetic map construction in barley (Wenzl et al. 2004) and some other crops (Lezar et al. 2004; Xia et al. 2005; Wittenberg et al. 2005; Akbari et al. 2006).

As an initial step toward synthesizing the genetic information available for barley based on DArT markers, the present study integrates the linkage data from four independent populations into a single consensus map. This consensus map could serve as a reference genetic map for barley, as a basis for studies of genome organization and evolution and a tool for molecular breeding in barley based on DArT markers.

This consensus map was used to study segregation distortion. Marker segregation distortion is an interesting phenomenon often encountered during the course of linkage map construction and has been defined as a deviation of the observed genotypic frequencies from their expected values which violates the Mendelian law of segregation. If a gene that causes segregation distortion is segregating in a population, then markers close to it would also tend to exhibit distorted ratios (Zamir and Tadmor 1986). If several populations have the same gametophyte factors or unknown genes that cause the segregation distortion, then these populations will exhibit segregation distortion in the same chromosomal regions. Molecular marker analysis in several populations is therefore useful for finding common regions with segregation distortion (SDRs) and for future identification of yet-unknown genes that cause segregation distortion in these regions (Lu et al 2002). There has been no comparative study of segregation distortion in different barley populations. The objectives in this study were to assess the frequency of occurrence of segregation distortion in barley and identify chromosomal regions consistently associated with segregation distortion.

5.2 Materials and Methods

5.2.1 Populations and markers

The four mapping populations used in this study are described in Table 5.1. The first population consisted of 94 doubled haploid lines from a barley cross between TX9425 and Franklin, as used in Chapter 4. This population was produced by the microspore culture technique described by Davies et al. (1998; 2003).

Table 5.1 Populations and markers assayed (before deleting redundant markers or DH lines).

Population	Type	DH method	Size	'bPb' DArT markers	'bPT' DArT markers	Other markers
Franklin/TX9425 (F/T)	DH	Anther culture	94	412	–	28
Franklin/Yerong (F/Y)	DH	Anther culture	188	496	–	22
Clipper/Sahara (C/S)	DH	*Hordeum bulbosum*	94	634	–	301
Steptoe/Morex (S/M)	DH	*Hordeum bulbosum*	94	746	539	215

The second populations consisted of 188 doubled haploid lines from the barley cross between Yerong and Franklin, again described in Chapter 4. This population was produced by the microspore culture technique described by Broughton (2002).

The third population was developed by Adelaide University from a cross between the Australian variety Clipper and the Algerian landrace Sahara 3771. The population (150 DH lines) was produced by the *Hordeum bulbosum* method described by Islam and Shepherd (1981), using embryo culture followed by chromosome doubling through

colchicine treatment. Linkage maps based on RFLP and SSR markers have been published by Karakousis et al. (2003). 94 DH lines from this population were genotyped using DArT technology. The original data for this population was provided by DArT P/L.

The fourth population was developed by the Oregon State University Barley Breeding Program for the North American Barley Genome Mapping Project (NABGMP). The parentage of the population is "Steptoe"/ "Morex". A population of 310 DH lines was developed from the F_1 by the *Hordeum bulbosum* technique, as described by Chen and Hayes (1989). A linkage map of this population has been published by Kleinhofs et al. (1993) and Wenzl et al. (2004). 94 DH lines genotyped for DArT markers were made available for this project. The original data for this population was provided by DArT P/L.

All four populations were genotyped with an identical set of DArT markers from a *PstI/Bst*NI representation ('bPb' markers). The Steptoe/Morex population was also assayed with a second set of DArT markers from a *PstI/Taq*I representation ('bPt' markers) (Table 5.1). Preparation of the DArT markers was carried out in the laboratory at DArT P/L.

5.2.2 Statistical analysis

5.2.2.1 Segregation analysis

Mendelian segregation was tested by Chi-square goodness-of-fit to a 1:1 ratio at a 0.5%, 1% and 5% significance level using JoinMap 3.0 (Van Ooijen and Voorrips 2001). . The presence of a segregation distortion region (SDR) was declared where three or more closely linked markers exhibited significant segregation distortion in one or more of the four populations.

5.2.2.2 Linkage analysis and map integration

Construction of component maps

A total of 18 DArT assays had to be discarded because a subset of DNA samples became contaminated (these samples could be identified because of their bias toward "1" scores) or because the relative hybridization intensities of non-polymorphic DArT markers were

not sufficiently correlated with the corresponding intensities in simultaneously performed assays (average correlation coeffiecient < 0.80). The presence vs. absence DArT scores (0/1) of the remaining 456 DH lines were converted into genotype codes (A/B/C/D) by comparison with the appropriate parental DArT assays (7 in total). Some markers for which both parental assays produced unreliable data were arbitrarily assigned to one of the two linkage phases. A few redundant DH lines were identified with Joinmap 3.0 using a similarity threshold of 95%. They were removed from the datasets, thus reducing the total number of lines to 454. The segregation data for DArT markers were merged with those of other markers for each population.

The segregation signatures of each of the four individual datasets were imported into JoinMap 3.0 to distribute loci into linkage groups. The LOD threshold used to define linkage groups was necessarily dependent on the number of markers and DH lines in the datasets. Markers in the wrong linkage phase were identified and flipped into the opposite phase. The known chromosomal locations of a subset of the DArT markers (Wenzl et al. 2004) were used to assign linkage groups to chromosomes in the Clipper/Sahara population. For the other three populations, the published SSR or RFLP markers were used to assign linkage groups to chromosomes.

Construction of a consensus map

A dataset containing 2425 DArT, 205 RFLP, and 346 SSR markers that were mapped in at least one of the four populations was used for map integration. The four datasets were loaded into the Joinmap 3.0 project (Van Ooijen and Voorrips 2001), pairwise estimates of θ and corresponding LOD scores were calculated from segregation data for each population. When orthologous markers defined an interval for two or more populations, Joinmap replaced the individual values of recombination fraction (θ) with a weighted average value (Stam 1993). The linkage groups that related to the same chromosomes across all four populations were combined by applying the *Combine Groups for Map Integration* function. The pairwise recombination frequencies between markers of the selected groups were combined into a new combined group node. The order of the markers in the combined group was calculated using a weighted average θ value one chromosome at a time. Map figures were exported using the computer program MapChart (Voorrips et al. 2002).

5.3 Results

5.3.1 Linkage map in Clipper/Sahara population

A genetic linkage map was constructed in population Clipper/Sahara based on 88 progenies (Fig. 5.1). A total of 935 markers (634 DArTs and 301 SSRs) were screened in this population (Table 5.1). After removing all redundant markers, 815 markers (522 DArT and 293 SSR) were used in linkage analysis and ten linkage groups were defined at a LOD threshold of 5.5 (Table 5.2). Markers were mapped to 357 positions covering a total map distance of 1073 cM. The ten linkage groups were assigned to the seven

Table 5.2 Various statistics associated with the four component maps.

	C/S	S/M	F/T	F/Y	Mean± SD
Number of DH lines used in each population	88	94	92	180	454
Number of loci	815	1,232	450	476	728
Number of bins (positions)[a]	357	508	214	262	335
Inter-bin distance (cM)					
Average	3.0 ± 2.7	2.2 ± 2.7	5.2 ± 4.6	4.1 ± 3.6	3.7 ± 1.1
Median	2.0	1.2	3.2	1.5	2.0 ± 0.8
Map length (cM)	1,073	1,093	970	1,072	1,052

[a] Co-segregating loci were collapsed into bins (unique loci) at the population-level, i.e. without concatenating segregation signatures across populations.

chromosomes by using a subset of the DArT markers with known chromosomal locations (Wenzl et al. 2004). Chromosome 5H, 6H and 7H each split into two segments. The distribution of the markers over the map was good except for several gaps on chromosome 4H and 5H. The average distance between bins was 3.0 cM and the largest gap between two bins was 19.3 cM.

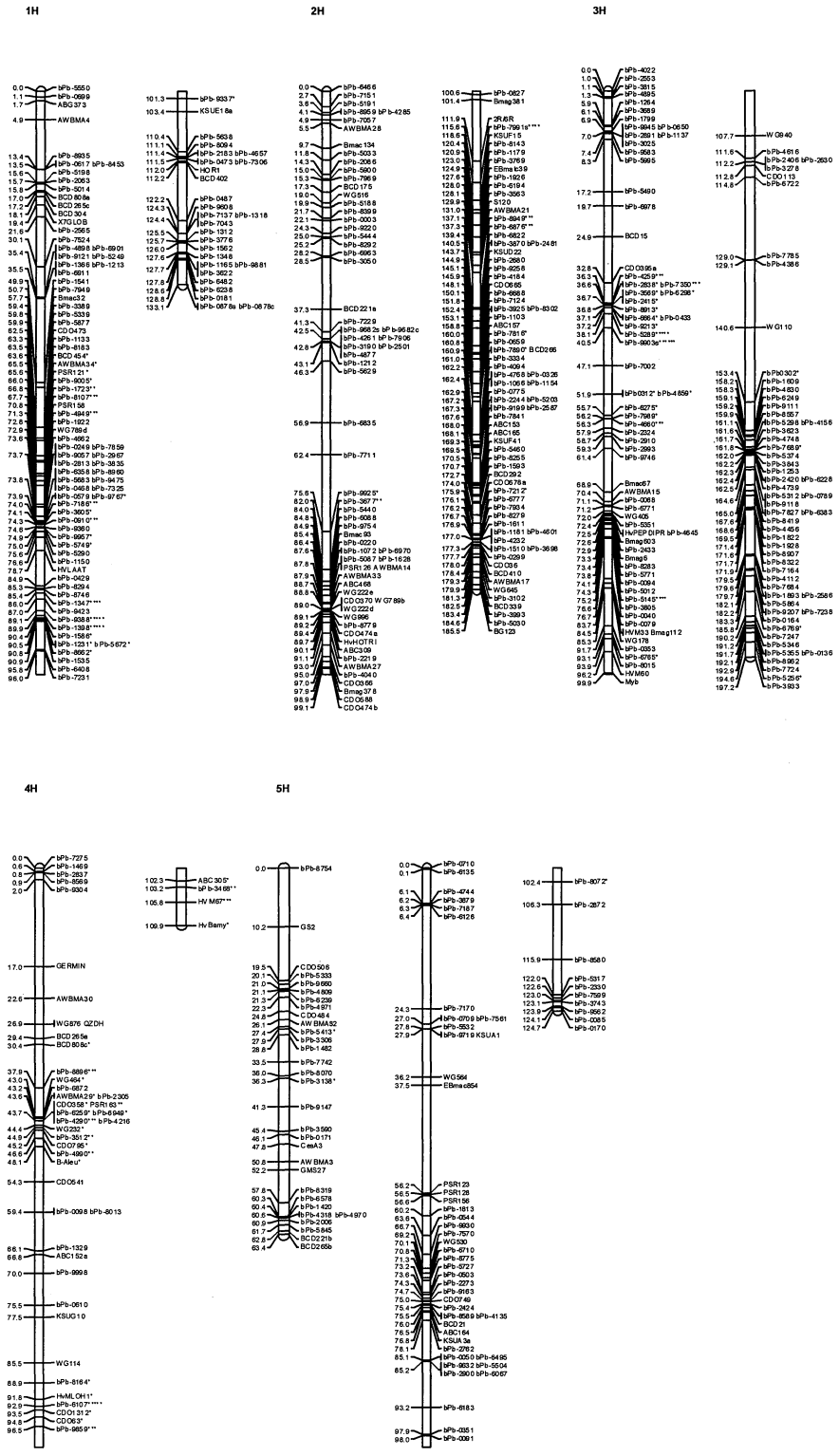


Fig. 5.1 Linkage map of the Clipper/Sahara population, showing the markers with segregation distortion. Enlarged version of this Figure is in appendix 4.

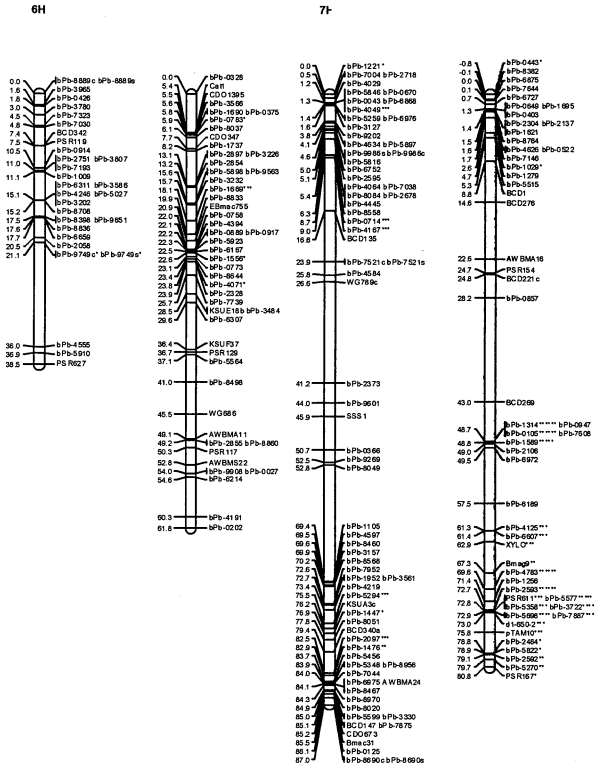


Fig. 5.1 continued

5.3.2 Linkage map in Steptoe/Morex population

A genetic linkage map was constructed in the Steptoe/Morex population based on 94 progenies (Fig. 5.2). A total of 1500 markers (1285 DArTs, 205 RFLPs and 10 SSRs) were screened in this population (Table 5.1). After removing the redundant markers, 1234 markers (1022 DArT, 202 RFLP and 10 SSR markers were used in the linkage analysis, and eight linkage groups were defined at a LOD threshold of 5.5 (Table 5.2). Markers were mapped to 508 positions covering a total map distance of 1093 cM. The eight linkage groups were assigned to the seven chromosomes by using RFLP and SSR markers as the anchor markers, with chromosome 5H split into two linkage groups. The distribution of the markers over the map was good except for several gaps on chromosome 2H, 4H and 5H. The average distance between bins was 2.2 cM and the largest gap between two bins was 17.1 cM.

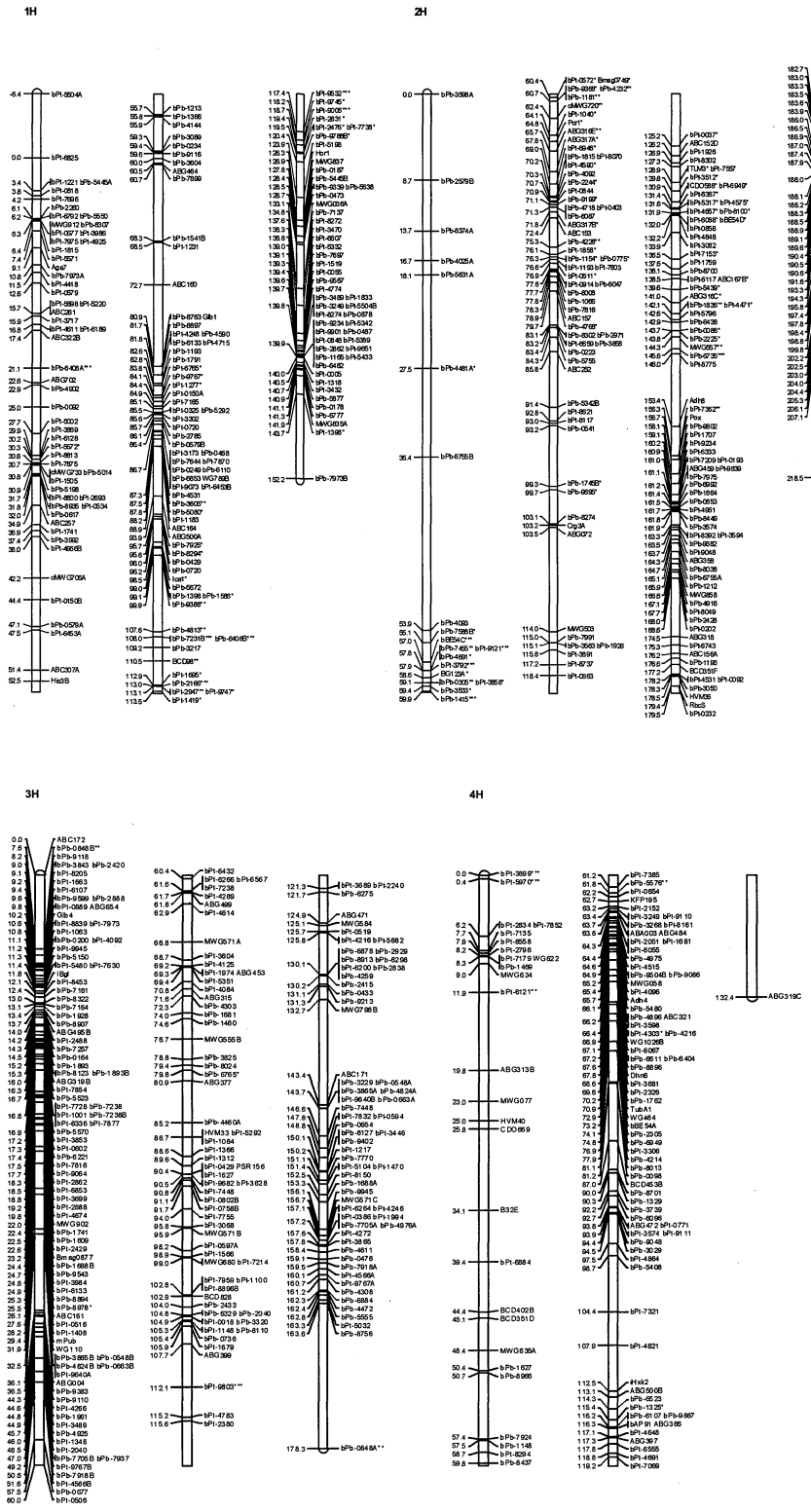


Fig. 5.2 Linkage map of the Steptoe/Morex population, showing the markers with segregation distortion. Enlarged version of this Figure is in appendix 5.

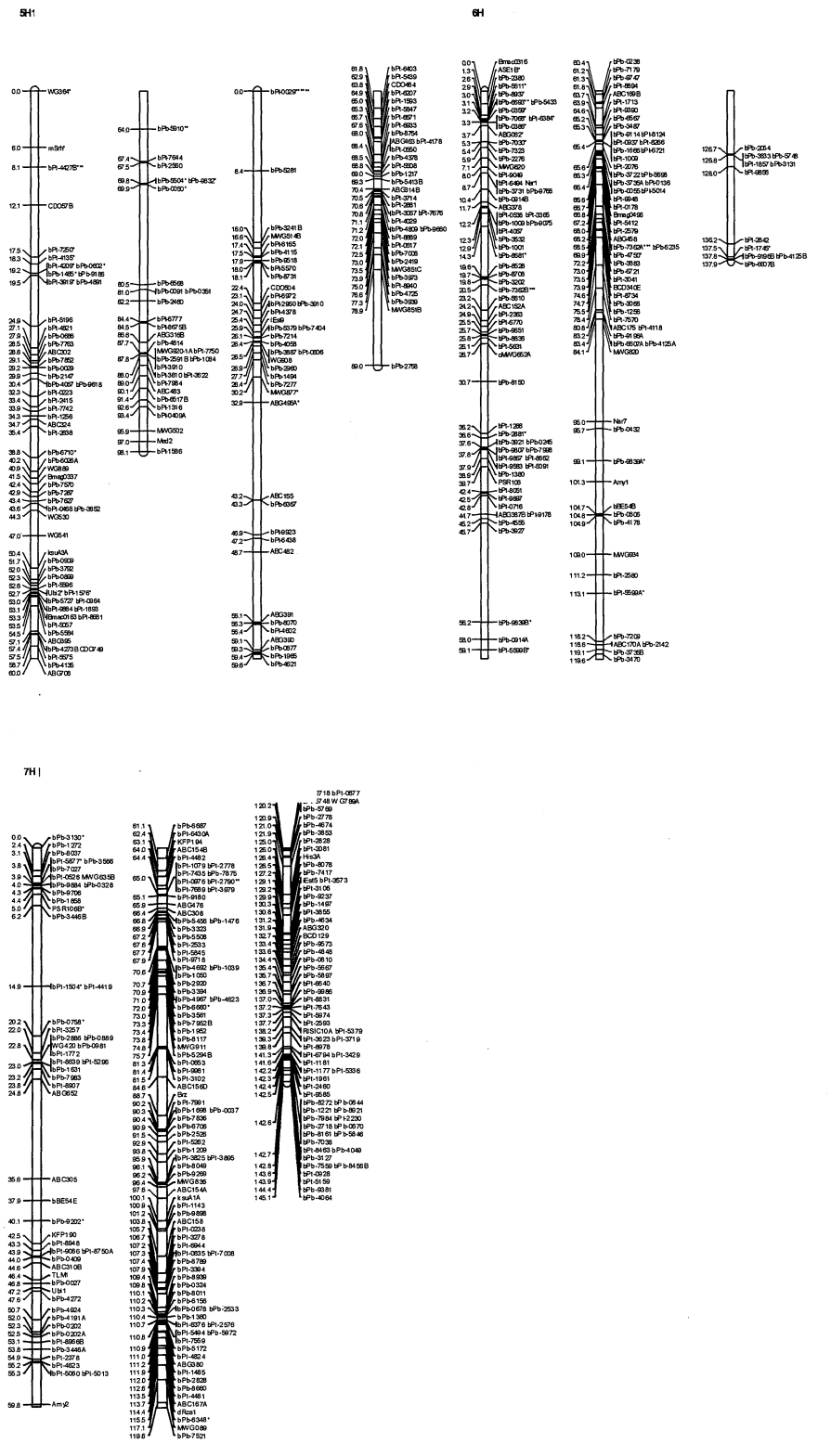


Fig. 5.2 continued

5.3.3 Co-linearity between each individual map

All the maps used the 'bPb' set of DArT markers and some of these markers were polymorphic in more than one population. These orthologous markers were compared for their colinearity among linkage group homologues (Fig.5.3). With minor exceptions, a high level of colinearity was observed among the orthologous markers of these linkage groups. The minor exceptions included some groups such as in the top parts of chromosomes 1H and 7H between C/S and S/M population, for which, although markers mapped to the same general area as those from other linkage group homologues, the marker order was slightly different. These regions contained a relatively high frequency of double crossovers that made conclusive ordering of markers difficult.

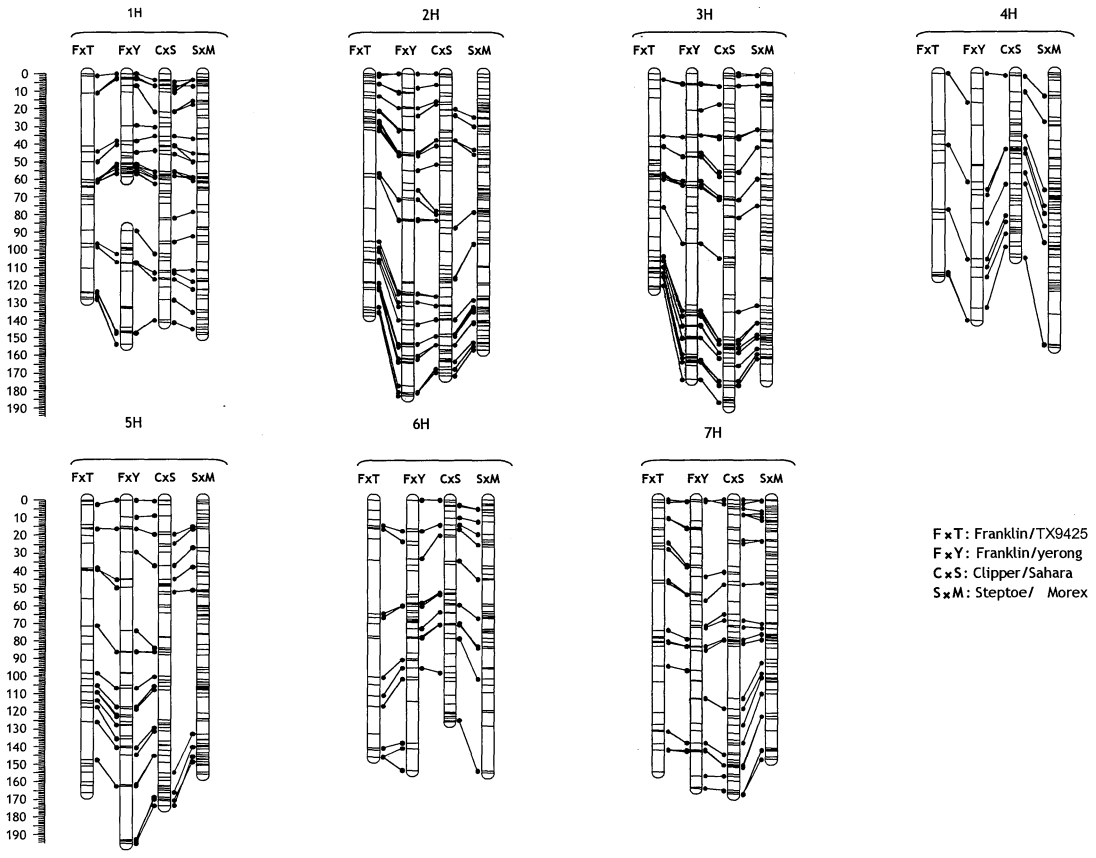


Fig.5.3. Colinearity of locus order in each component maps. Loci in component maps were displayed schematically by horizontal lines across the bars representing each chromosome. Loci that were common between adjacent pairs of populations are depicted by dots and connected by lines. The scale on left side is in cM.

5.3.4 A consensus map from the combined datasets

A consensus map was built (Fig.5.4) with JoinMap using a set of quality-filtered markers under conditions that were likely to minimize the number of misplaced loci (Table 5.2). A set of 2425 DArT, 205 RFLP, 346 SSR markers was selected for this purpose. Among these markers, the set of 'bPb' DArT markers was assayed across all populations and contained many good-quality anchor markers that bridged the four populations. RFLP, SSR and a second set of 'bPt' DArT markers were predominantly assayed in one or two populations only. However, nearly a quarter of the 'bPb' DArT markers segregated in two or more crosses and a significant number of them in three or more. Within the populations in which they were polymorphic, the vast majority of the markers were scored with less than 10 missing data.

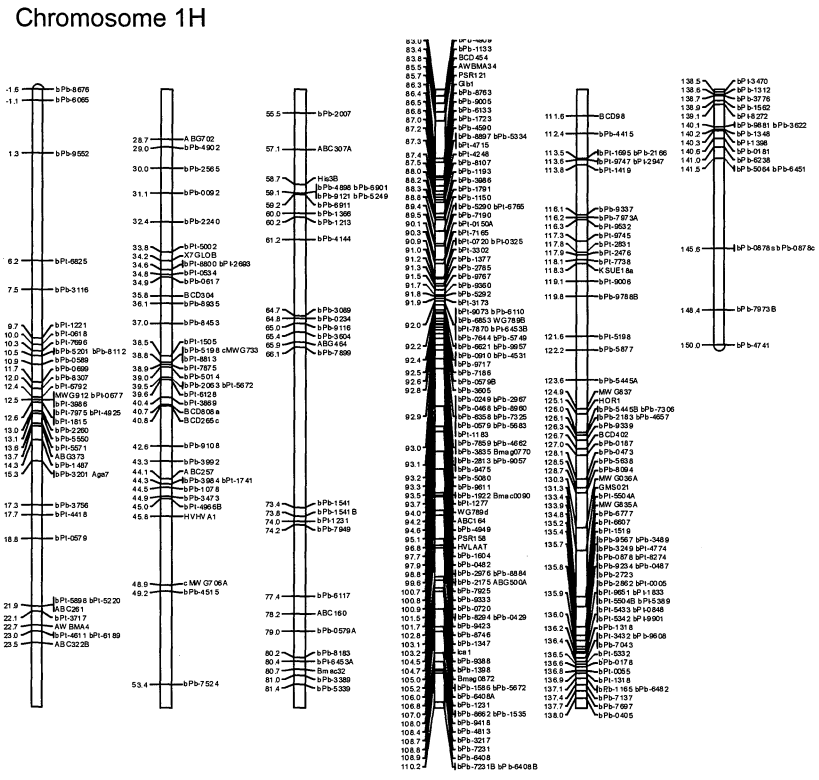
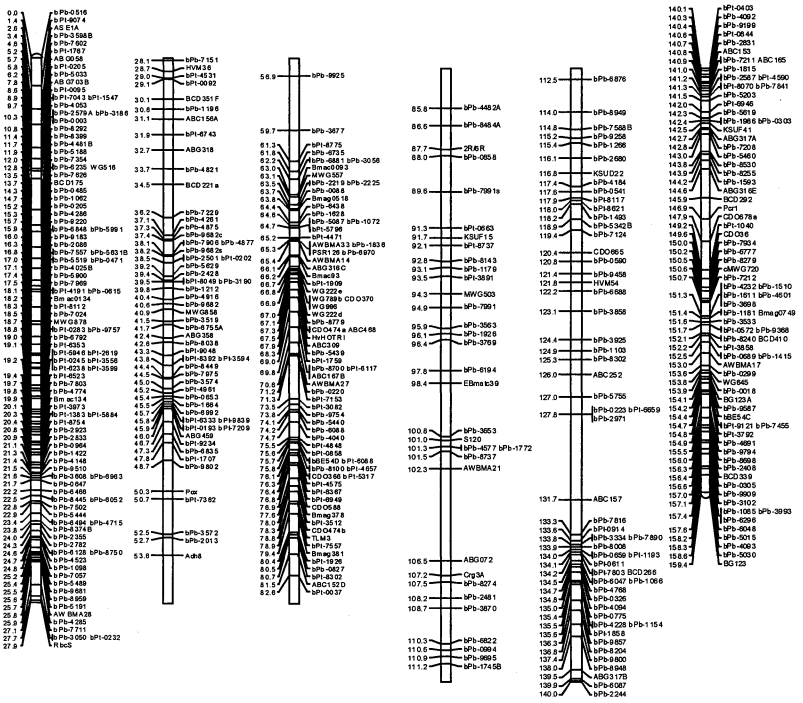


Fig. 5.4 A new barley consensus map based on mapping populations of Franklin/Tx9425, Franklin/Yerong, Clipper/Sahara, and Steptoe/Morex. Enlarged version of this Figure is in appendix 6.

Chromosome 2H



Chromosome 3H

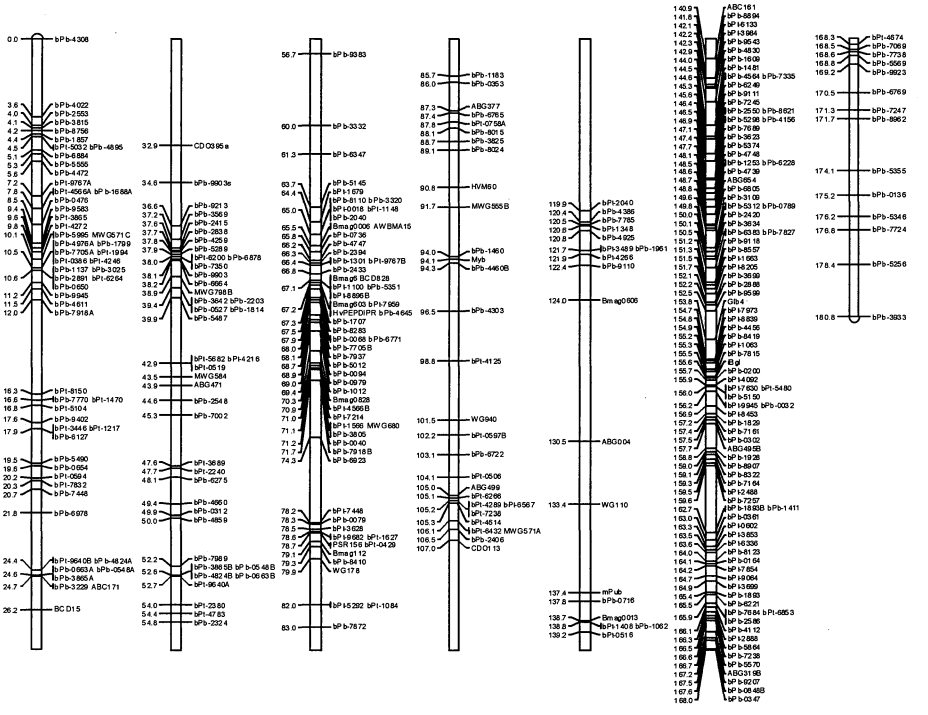
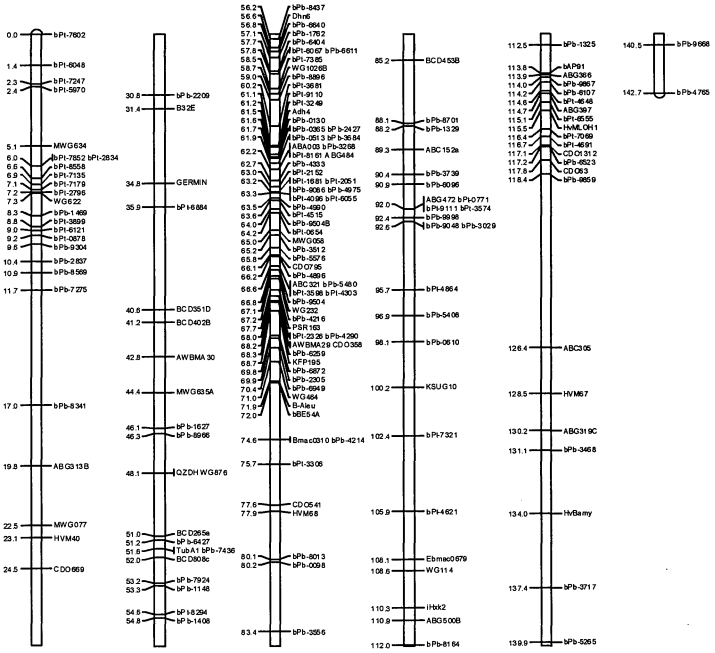


Fig. 5.4 continued

Chromosome 4H



Chromosome 5H

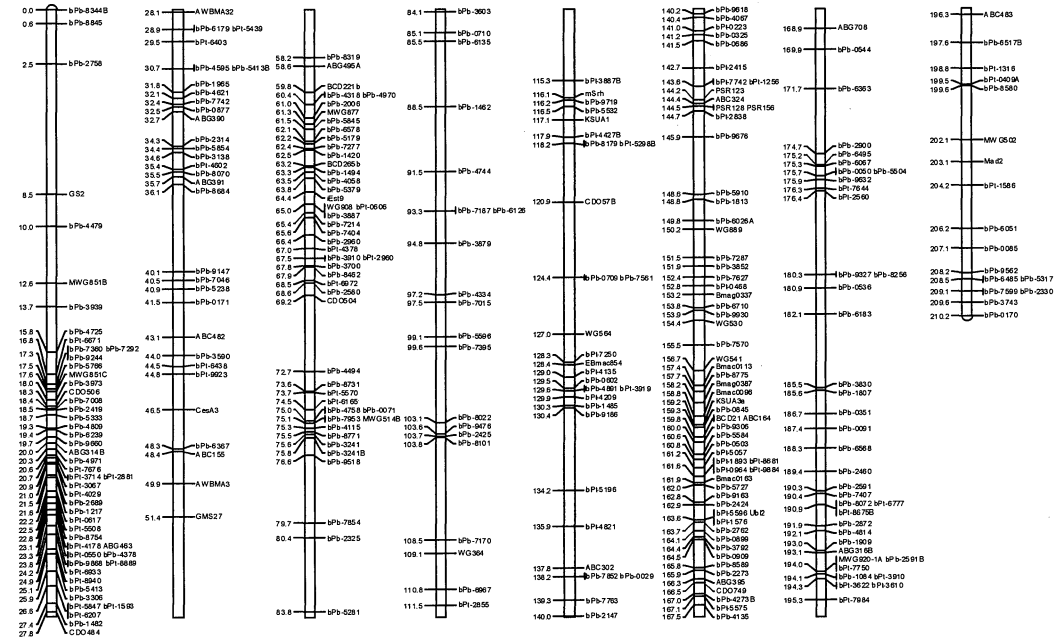
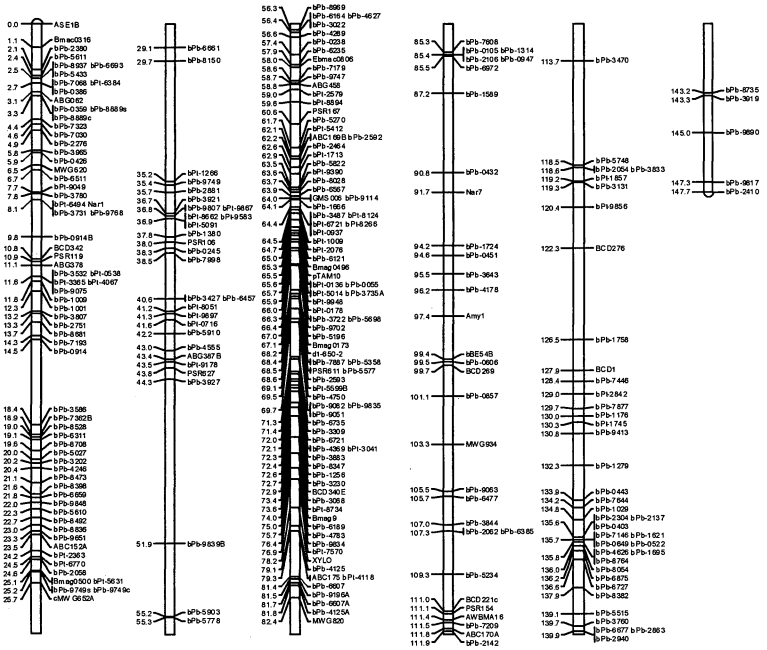


Fig 5.4 Continued

Chromosome 6H



Chromosome 7H

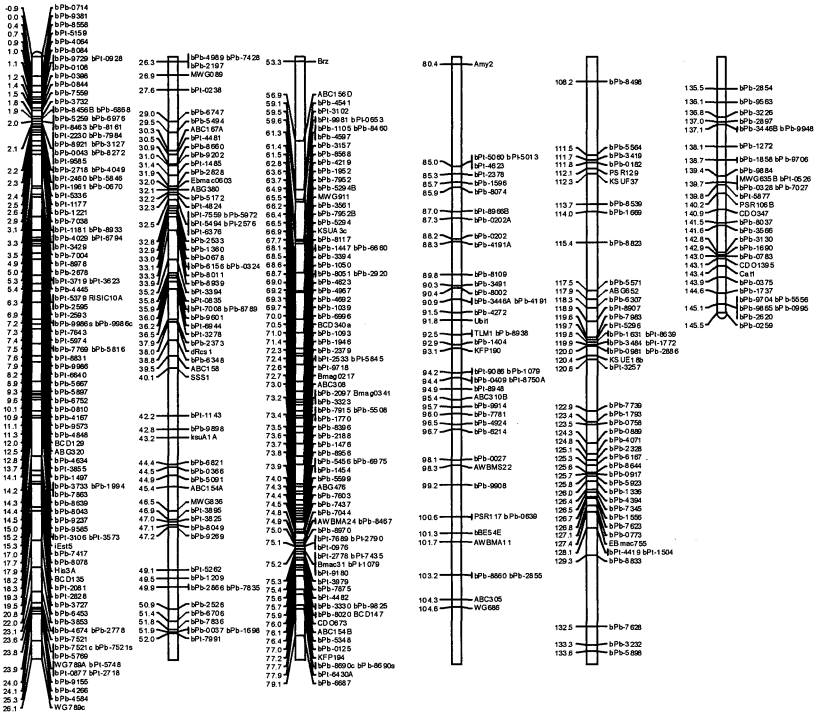


Fig. 5.4 continued

Table 5.3 Statistics of consensus map by chromosome.

	1H	2H	3H	4H	5H	6H	7H	Genome
Number of loci of each type								
All	302	403	309	162	294	266	375	2,111
DArT	259	313	271	108	238	217	321	1727
'bPb' DArT	180	233	190	66	175	175	241	1260
Others	43	90	38	54	56	49	54	384
Map length (cM)								
All	150.0	159.4	180.8	142.7	210.2	147.7	145.5	1,136
DArT	150.0	158.6	180.8	142.7	210.2	145.6	145.5	1,133
'bPb' DArT	150.0	158.6	180.8	134.4	210.2	145.6	145.5	1,125
Others	121.4	156.8	157.1	128.9	194.6	127.9	137.1	1,024
Average/median inter-loci distance (cM)								
All	0.5/0.3	0.4/0.3	0.6/0.3	0.9/0.4	0.7/0.5	0.6/0.3	0.4/0.3	0.5/0.3
DArT	0.6/0.4	0.5/0.3	0.7/0.3	1.3/1.0	0.9/0.6	0.7/0.4	0.5/0.3	0.7/0.4
'bPb' DArT	0.8/0.4	0.7/0.4	1.0/0.3	2.0/1.3	1.2/0.7	0.8/0.4	0.6/0.3	0.9/0.4
Others	2.8/1.3	1.7/1.0	4.1/2.6	2.4/1.0	3.5/1.9	2.6/1.7	2.5/1.4	2.7/1.6

* Map features were calculated for the whole dataset and various subsets obtained by removing selected types of loci while maintaining the locus order of the consensus map.

The final consensus map comprised of 2,111 markers arranged into seven linkage groups, corresponding to the seven chromosomes. This number is considerably larger than the number of markers in previously published consensus maps (587–1,536) (Langridge et al. 1995; Qi et al. 1996; Karakousis et al. 2003; Diab et al. 2006). On average, each chromosome contained 246 DArT and 55 non-DArT loci. The number of DArT loci per chromosome ranged from 108 (4H) to 321 (7H). The number of non-DArT loci ranged

from 38 (6H) to 90 (2H) (Table 5.3). The consensus map spanned a total length of 1,136 cM. Chromosome sizes ranged from 142.7cM (6H) to 210.2 cM (5H) (Table 5.3; Fig.5.3). The 'bPb' DArT markers alone spanned 99.0% of the total length of the consensus map. Addition of a second set of DArT markers ('bPt' markers) increased coverage to 99.7%. The combination of all non-DArT markers resulted in a coverage of 90.1% (Table 5.3). The DArT subset of markers generated a consensus map with a single gap between 15 and 20 cM (3H) and three gaps between 10 and 15 cM on chromosomes 3H and 4H. The DArT markers were a good complement to the other markers since they added the map length and filled many gaps. The map had only a single gap larger than 10 cM (3H) and ten other gaps between 5 and 10 cM (1H, 3H, 4H, 5H, and 6H).

The average resolution of the consensus map was evaluated by calculating the average distances between adjacent markers. The 2,111 markers of the whole dataset were distributed through the barley genome with an average inter-marker distance of 0.54 ± 0.81 cM (median = 0.34 cM). This resolution was only moderately greater than the resolution obtained with DArT loci alone (0.66 ± 1.1 cM; median = 0.43 cM). The set of 'bPb' DArT markers, which were simultaneously assayed in a single reaction, provided a resolution of 0.89 ± 1.40 cM (median = 0.44 cM). Non-DArT markers on their own produced a map with a resolution of 2.66 ± 3.03 cM (median = 1.57 cM; Table 5.4).

5.3.5 Comparison with component maps

An alternative way to evaluate the quality of a consensus map is to compare the locus arrangement of the consensus map (optimized at the multi-population level) with the arrangement of loci in the component maps (each one optimized separately).

To quantify the consistency of locus order between the two different types of maps, unique loci of each of the four datasets were alternatively arranged according to the consensus or the component map to compute two alternative sets of locus positions per dataset. The Pearson correlation coefficients for the alternative sets of locus positions ranged from 0.9998 ± 0.0003 (1H) to 0.99996 ± 0.00006 (3H) (means ± SD across four populations). The order of loci in the consensus map therefore can be concluded to properly reflect the arrangement of loci in the individual component maps.

As a separate indicator of the quality of the consensus locus order, the degree to which

component maps expanded if their loci were arranged according to their order in the consensus map could be quantified. Chromosome lengths computed with the algorithm of JoinMap 3.0 hardly showed any expansion: 0.34 ± 0.43% (mean ± SD across populations). The sum of adjacent recombination fractions (SARF), a more sensitive indicator of map expansion caused by suboptimal marker positioning, revealed a minor degree of expansion of 5.2 ± 2.9% (mean ± SD across populations). This is not surprising because some residual genotyping errors can cause an incorrect locus order to appear superior to the correct order, which can happen more easily if only the segregation data of a single population are taken into account.

Both the indicator of locus order consistency and the degree of map expansion were closely associated with the fraction of DArT loci in the component datasets. Datasets dominated by DArT markers showed more favourable values. This trend probably reflects the fact that non-DArT markers were, on the average, assayed in fewer populations than the DArT markers. Their positions on the consensus map, therefore, were more ambiguous, particularly if they were located in regions where component maps differed in length. Any (hypothetical) error in DNA sample tracking between DArT and non-DArT marker assays would have introduced artificial crossovers which may have differentially impacted on the accuracy of locus ordering in component maps and the consensus map.

5.3.6 Detection of marker segregation distortion and comparison of segregation distortion regions (SDRs) based on the consensus map.

Marker segregations were tested for deviation from the expected Mendelian segregation (1:1) by chi-squared analysis. The statistics of marker segregation distortion for the four mapping populations used in this study were described in Table 5.4 and Table 5.5. Among the 815 markers in the Clipper/Sahara population, 16.6% showed segregation distortion at the 0.05 level of significance, 7.36% at the 0.01 level and 5.94% at the 0.005 levels (Table 5.4). The markers with segregation distortion were not distributed randomly, for example, at 0.01 significance level, the distorted markers were mainly mapped to chromosome 1H, 3H, 4H and 7H, the other three chromosomes having only 14.9% of the distorted markers (Table 5.5). While in the population of Steptoe/Morex, among the 1234 markers, 12.17% showed segregation distortion at the 0.05 level of

significance, 3.37% at the 0.01 level and 1.48% at the 0.005 levels (Table 5.4). The markers with segregation distortion were not distributed randomly, for example, at the 0.01 level of significance, 20.0% and 41.4% of the distorted markers were mapped to chromosome 1H and 2H respectively (Table 5.5).

Averaged across all markers, the frequency of the maternal allele (50.4%) in the Steptoe/Morex cross was very similar to that of the paternal allele (49.6%). However, in the Clipper/Sahara cross, the maternal allele (54.07%) is significantly higher that in other three populations. Out of the 2975 markers used across all four populations, 635 (21.1%), 329 (10.9%), and 239 (7.9%) exhibited segregation distortion at 0.05, 0.01, and 0.005 respectively (Table 5.4). The lowest frequency of distorted markers was found in Steptoe/Morex population.

Table 5.4 Percentage of distorted markers in the four mapping populations tested at $p \leq 0.05$, 0.01 and 0.005. F/T = Franklin/TX9425, F/Y = Franklin/Yerong, C/S = Clipper/Sahara, S/M = Steptoe/Morex.

Populations	Rate of marker distortion			Number of distorted markers			Markers on each component map
	p ≤0.05	p ≤0.01	p ≤0.005	p ≤0.05	p ≤0.01	p ≤0.005	
F/T	0.409	0.272	0.202	184	122	91	450
F/Y	0.328	0.208	0.162	156	99	77	476
C/S	0.166	0.074	0.059	135	60	48	815
S/M	0.122	0.034	0.015	150	42	18	1234
Total	0.211	0.109	0.079	635	329	239	2975

Among the 635 markers showing aberrant segregation in the four populations, 459 (72%) markers were located in putative SDRs. Sixteen SDRs were identified (Fig. 5.4), and 10 of them were found in at least two populations. SDR 1.1 was detected in all four populations. The SDRs were identified on all seven chromosomes of barley, but they were unevenly distributed over the seven chromosomes. Most of the SDRs had consistent map locations over the four populations. SDR1.1, SDR2.1, SDR4.1, SDR5.1, SDR 6.2 and SDR7.1 had nearly identical map location for their most-severely distorted marker among the different populations. On the other hand, the size of SDRs varied from 4 cM in SDR3.3 to 46 cM in SDR5.3 (Table 5.5; Fig. 4) and between populations. Some of these SDRs, for example SDR 2.1 comprised three and SDR 5.2 and SDR 6.2 comprised two

smaller SDRs each. Further studies are needed to determine whether or not all these large SDRs comprise two or more smaller SDRs.

Table 5.5 Genome locations of segregation distortion regions (SDRs) identified in individual populations based on an integrated barley consensus map.

Chr.	F/T	F/Y	C/S	S/M
1H	88.0 - 108.0	98.8 - 105.0	83.8 - 87.5; 92.4 - 92.7; 104.5 - 105.2	104.5 - 108.7; 111.6 - 119.8
2H	58.6 - 59.4; 74.1 - 74.7; 114.0 - 121.8	56.9 - 64.7; 73.8 - 74.7		61.8 - 63.7; 65.2 - 69.8; 75.7 - 78.0; 144.6 – 155.4
3H	39.9 - 78.3; 124.0 - 157.5	162.7 - 166.7	34.6 - 38.2; 47.7 - 52.2	
4H	54.8 - 74.6		51.0 - 71.9; 112.0 - 134.0	
5H	75.6 - 99.6; 180.3 - 199.6	62.5 - 97.2; 153.2 - 199.6		109.1 - 117.9; 128.3 - 129.9
6H	101.1 - 136.0	4.4 - 11.8; 35.4 - 76.4	60.6 - 81.4; 85.4 - 87.2	
7H	44.4 - 76.4	68.5 - 76.4		

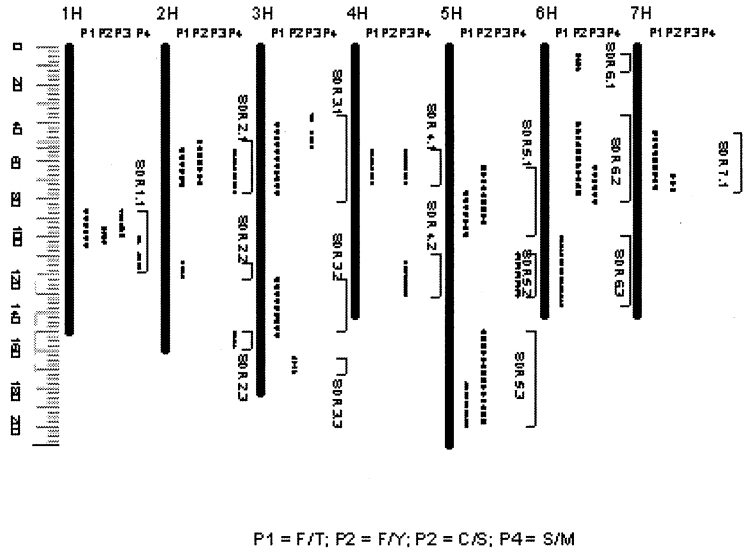


Fig. 5.5 Segregation distortion regions (SDRs) in four barley populations: P1 = Franklin/TX9425; P2 = Franklin/Yerong; P3 = Clipper/Sahara; P4 = Steptoe/Morex. Markers with segregation distortion in each individual population were re-located on the barley consensus map. The direction of each chromosome is the same as in Fig. 5.4.

5.4 Discussion

5.4.1 Construction of the consensus map

Mapping with multiple populations should provide several advantages over mapping based on a single population. In particular, a larger number of loci can be placed onto a single map, hence reducing the gaps between markers, and in particular avoiding separation of parts of chromosomes. This is especially important when attempting to map specific genes of interest (e.g., morphological markers or candidate genes for trait mapping) that are unlikely to segregate within a single mapping population. This also provides for great genomic coverage. These multi-population mapping studies have provided evidence for chromosome rearrangements (Beavis and Grant 1991; Kianian and Quiros 1992) and gene duplication (Kianian and Quiros 1992; Gentzbittel et al. 1995), have assisted in the assignment of linkage groups to chromosomes (Beavis and Grant 1991), and have provided the basis for comparative studies among related species and subspecies (Kianian and Quiros 1992; Hauge et al. 1993; Gentzbittel et al. 1995).

The present study integrates the linkage data from four independent populations into a single consensus map. Because the primary goal for the construction of this consensus map was to place, relative to one another, as many molecular markers as possible onto a single map, the concern in this study was more towards obtaining a general order among these markers rather than the fine resolution of order. Some changes in marker order (other than those due to translocation) were observed during construction of other consensus maps (Sewell et al. 1999; Lespinasse et al. 2000; Sebastian et al. 2000; Cervera et al. 2001; Jeuken et al. 2001; Lombard and Delourme 2001). It is believed that small discrepancies in marker ordering may be due to mapping imprecision rather than real rearrangements (Lombard and Delourme 2001). Some small differences in marker order were observed in the present work between the four individual linkage maps and the consensus map. One of the reasons for the discrepancies might be due to chance, because LOD score criteria may not be stringent.

The consensus map built in this study co-locates DArT markers with previously mapped SSR and RFLP markers. It provides a framework for transferring genetic information between different marker systems and for deploying DArT markers in molecular breeding schemes. It is not practical or necessary to construct very high density genetic

maps to identify the genomic locations of QTLs for an individual trait (Sewell et al. 1999). Thus QTL studies may be done in 2 phases, one where marker density is low, and the second where marker density is increased around putative QTL based on knowledge from consensus maps such as the one constructed here. In this study, QTLs relating to waterlogging tolerance in barley will be mapped in the two different mapping populations (see Chapter 6). It should be possible to summarize QTLs from different pedigrees on the consensus map and obtain knowledge of how many QTLs exist and which QTLs are expressed through the pedigrees in future analysis.

This study has also highlighted an increasing mismatch between our ability to rapidly genotype a large number of mapping populations and the performance of available software tools to construct a consensus map. Although Joinmap software was widely used for building genetic linkage maps and constructing consensus maps (Stam 1993; Van Ooijen et al. 2001), problems with using this program to analyze high-density datasets have been encountered (Isidore et al. 2003; Van Os et al. 2005; Wenzl et al. 2006). The problem encountered in this study is the huge computing time taken to work with high-density datasets. It took thousands of hours to construct the consensus map reported here. While from a statistical point of view it is preferable to build a consensus maps *de novo* from the integrated set of segregation data, it currently appears preferable to build a synthetic map from separately constructed component maps instead due to a lack of powerful programs (Wenzl et al. 2006), at least until improved or alternative software options become available.

5.4.2 Segregation distortion

As has been demonstrated in Chapter 4, there is no evidence that the segregation distortion was caused by genotyping and scoring errors using the DArT markers, since they suffered a rate of distortion that was lower than the SSR and AFLP markers.

A significant proportion of distorted markers, however, were mapped to particular chromosomal regions. Even in populations with a low segregation distortion rate, the distorted markers were not evenly distributed across chromosomes, for example, at the 0.01 significance level, 3.4% of the total markers showed distorted segregation in the Steptoe/Morex population, but 61.4% of these distorted markers were mapped to chromosomes 1H and 2H. This phenomenon indicates that segregation distortion in

barley is most likely to be caused by genetic factors.

Comparison of the rate of segregation distortion among the four populations showed that populations developed by androgenesis (microspore culture technique) had a much higher distortion rate than those developed by intergeneric hybridization (*Hordeum bulbosum* method in this study). A major limitation of androgenesis is the effect of the barley genotype on haploid plant production, this being in agreement with the previous reported studies (Devaux, et al. 1995).

Many genes are expressed postmeiosis during microspore and pollen development in angiosperms (Mascarenhas 1992), thus if any of these genes are variable there may be selection. Alternatively, segregation distortion could have arisen from hybrid sterility genes that caused the abortion of specific gamete or zygote genotypes. These partial lethal factors may make an androgenetic plant population show an increased rate of segregation distortion. Two regions on chromosomes 1H and 5H in anther-derived DHs from the F_1 between Steptoe and Morex showed strong distortion (Devaux et al. 1995). Similarly, distorted loci were reported in DH populations from other crosses on chromosomes 1H (Kintzios et al. 1994), 5H (Steffenson et al. 1995), 4H and 6H (Thompson et al. 1991), 2H and 7H (Logue et al. 1995) and 3H (Graner et al.1991; Devaux et al. 1995).

Mukai et al. (1995) speculated that distorted segregation of these markers was caused by putative "embryonic lethal gene(s)" (or viability genes), because clustering of genetic markers showing distorted segregation ratios is consistent with the idea that they may be closely linked to a viability gene. It is necessary to compare segregation distortion among several populations that are segregating for the same gametophyte factors or other unknown genes that cause segregation distortion, as these populations will exhibit segregation distortion at the same chromosomal regions. Molecular-marker analysis in several populations is therefore useful for finding common regions with segregation distortion (i.e., segregation distortion regions or SDRs) and for future identification of yet-unknown genes that cause segregation distortion in these populations.

The segregation distortion regions (SDRs) in barley might be linked to a group of genes involved in the process of haploid production. For example, there may be differences in barley genotypes responding to microspore culture and the frequencies of green haploid

plant production. Likewise the crossability of barley × *H. bulbosum* may depend on the barley allelic composition for the genes with functions like those of Kr genes in wheat (Snape et al. 1979; Falk and Kasha 1983). Some common genes are expressed in all the populations, while others are just expressed in some specific crosses.

Chapter 6 Identification and characterization of quantitative trait loci (QTLs) associated with waterlogging tolerance in barley

6.1 Introduction

Quantitative trait loci (QTL) represent regions of the genome that have a measurable effect on variation in a particular trait. QTL analysis essentially calculates a statistical association between the phenotypic trait of interest and segregating genetic markers. The core idea of QTL mapping is no different to Mendelian recombination mapping, except that multiple loci, none of which is solely responsible for the trait, can be mapped simultaneously. Instead of looking for perfect association between a marker and the phenotype, the genome is scanned for statistically significant associations between markers and the phenotype (Members of the Complex Trait Consortium 2003).

The key features of successful QTL mapping designs are the ability to control the starting genetic variance; to reduce the environmental variance (in order to increase the proportion of overall phenotypic variance that is due to each QTL); and to increase the number of meiosis divisions as desired. It is a powerful method of studying the effects of individual loci that contribute to the expression of a quantitative trait. Such loci cannot be investigated individually using classical Mendelian genetics as their effects are usually individually small and get lost in the background of other variation (Falconer and Mackay 1996).

Relatively high-resolution QTL mapping was enabled in the 1990s by the development of new techniques for marker genotyping (Asins 2002). Initially, significance was judged by t-test for the difference between the mean phenotypes of the two allele classes at each marker. However, this procedure underestimated the magnitude of QTL effects, was unable to separate closely linked QTLs and had low precision. The development of novel statistical methods for QTL detection has accelerated the progress of QTL mapping. The interval mapping (IM) procedure (Lander and Botstein 1989) improved resolution and power by estimating marker genotypes and association at each position in the interval

between adjacent pair of markers. Subsequent modifications included conditioning of a marker's effects on other significant markers in the genome (CIM-composite interval mapping; Zeng 1993; 1994) and simultaneous fitting of multiple QTL effects (MQM-multiple QTL model; Jansen 1993; 1994). Other alternative methods for identification of QTLs such as transposable element insertional mutagenesis and mapping the haploid sufficient effects of small deletions (also known as deficiency complementation mapping; Long et al. 1996) are likely to merge with interval mapping methods and functional genomics in the coming years to resolve the molecular basis for quantitative traits to levels that could barely be imagined in the pre-genomic era.

Conventional QTL detection depends on the segregation of a marker allele with trait values above (or below) the average trait value. However across a number of environments, QTLs with strong environmental interaction may not be detected using this method. Where there are two or more QTLs detected for a trait, two-way interactions among the QTLs identified for each trait cannot be detected in IM and CIM methods. In IM and CIM methods, all regression effects are fixed, they cannot handle complex effects by regression model, for example, they do not include random effects of E & QE (environment and QTL by environment interaction). A mixed-model-based composite interval mapping (MCIM) has been developed (Zhu 1999; Wang et al. 1999) which can handle both fixed and random effects. For example it can estimate fixed QTL effects and random QE effects with no bias and map QTLs with additive or epistatic effects (additive x additive), as well as their interactions with the environment. Recently, this model has been updated by adding dominance effects, epistatic effects of additive × dominance and dominance × dominance as well as their interaction with the environment (Yang et al. 2005; Gao et al. 2006). QTL analysis has proven to be very useful in identifying the genetic components of the genetic variation for important economic traits (Mazur et al. 1995). For example, numerous genes of economic importance, such as those for disease and insect resistance, are repeatedly transferred (or backcrossed) from one varietal background to another by plant breeders (Hayward et al. 1993). Most genes behave in a dominant and recessive manner and require time-consuming efforts to transfer. Sometimes the screening procedures are cumbersome and expensive and require large field space. If such genes can be tagged by tight linkage with molecular markers, time and money can be saved when transferring them from one varietal background to another. A

molecular marker very closely linked to the target gene can act as a "tag" which can be used for indirect selection of the gene(s) in a breeding programme (Khush et al. 2004).

Due to the availability of a large number of genetic stocks and its considerable economic importance, barley has been proposed as a model for the entire *Triticeae* on genome mapping (Linde-laursen et al. 1997) and great progress has been made in QTL mapping economically important traits in barley, including malting quality (Thomas et al. 1996; Han et al. 1997; Bezant et al. 1997; Swanston et al. 1999; Marquez-Cedillo et al. 2000), agronomic traits (Backes et al. 1995; Thomas et al. 1995; Laurie et al. 1995; Bezant et al. 1996; Tinker et al. 1996; Borem et al. 1999; Marquez-Cedillo etal. 2000; Borner et al. 2002), development patterns (Karsai et al. 1997), yield and yield components (Kjar et al. 1996; Bezant et al. 1997; Yin et al. 2002), germination (Mano et al. 1997), seed dormancy (Romagosa et al. 1999), water stress related traits (Teulat et al. 1997), osmotic-adjustment (Teulat et al. 1998), enzyme activities (Han et al. 1995; Borem et al. 1999), starch granule trait (Borem et al. 1999), salt tolerance (Mano et al. 1997), boron toxicity tolerance (Jefferies et al. 1999), shoot differentiation ability (hackett et al. 1992; Komatssuda et al. 1993), head shattering (Kandemir et al. 2000), crossability with wheat (Taketa et al. 1998), winterhardiness (Hayes et al. 1992, Pan et al. 1994), and resistance to cereal aphids (Moharramipour et al. 1997), leaf stripe (Pecchioni et al. 1996; Pecchioni et al. 1999; Arru et al. 2002), leaf rust (Spaner et al. 1998; Graner et al. 2000; Park et al. 2002), stem rust (Spaner et al. 1998), stripe rust (Chen et al. 1994), net blotch (Steffenson et al. 1996; Spaner et al. 1998), powdery mildew (Heun et al. 1992; Backes et al. 1996; Spaner et al. 1998), spot blotch (Steffenson et al. 1996), Scald (Spaner et al. 1998; Garvin et al. 2000; Jensen et al. 2002), bacterial leaf streak (El Attari et al. 1998), barley yellow dwarf virus (Crasta et al. 2000), and fusarium head blight (Zhu et al. 1999; de la Pena et al. 1999).

Little progress has been made, however, in mapping waterlogging tolerance in barley because it is affected by many confounding factors such as temperature, plant development stage, nutrient levels, timing and duration of waterlogging, soil type and sub-topography (Setter et al. 2003). It is difficult to measure reliably in field experiments, and hence a lack of efficient screening methods makes it very hard to bring waterlogging tolerance into commercial barley varieties. The availability of a large numbers of molecular marker systems and many powerful computer programs for QTL analysis

should make screening easier. With recent research showing that leaf yellowing and chlorophyll fluorescence may be simple traits to use in the evaluation of waterlogging tolerance in barley (Pang et al. 2004), QTL identification has become possible for waterlogging tolerance in barley by mapping physiological traits associated with waterlogging tolerance (Qian et al. 2005). The characterization of QTLs for barley waterlogging tolerance is an important step in the genetic dissection of this trait. In order to achieve this target, characterization of QTLs controlling barley waterlogging tolerance related traits is the focus of this chapter.

6.2 Materials and Methods

6.2.1 Generation of phenotypic data

6.2.1.1 Populations used for QTL analysis

The first population consisted of 92 doubled haploid lines from the barley cross between TX9425 and Franklin, as discussed in Chapters 4 and 5. TX9425 is a feed barley with waterlogging tolerance introduced from China while Franklin is an Australian malting barley. The two parents are different in not only waterlogging tolerance but also many other traits, including malting quality, resistance to some diseases and agronomic traits (Dr Meixue Zhou, unpublished data). A linkage map based on 92 lines from this population was constructed using DArT, AFLP and microsatellite markers (Chapter 4 of this thesis).

The second population consisted of 182 doubled haploid lines from the barley cross between Yerong and Franklin. Yerong is an Australian six-rowed variety with good tolerance to waterlogging stress. A genetic linkage map was constructed using DArT and microsatellite markers, as discussed in chapters 4 and 5 of this thesis.

6.2.1.2 Evaluation of waterlogging tolerance of the DH lines

Germination trial

The germination experiment was performed with six replicates of fifty seeds for each DH line, three as control and three for waterlogging. For the control, in each replicate, fifty seeds were submerged in 50 ml of deionized water in tubes (25 x 120 mm) overnight (Fig. 6.1), and the seeds were then moved to a moist filter paper in 9 cm Petri dishes and

incubated at 25 °C for 8 days, then the seeds with emerging radicle or plumule were recorded. In the waterlogged treatment, three replicates of fifty seeds were submerged in 50 ml of deionized water in tubes (25 x 120 mm) for 6 days in an incubator at 25 °C (Takeda and Fukuyama 1987). After the flooding treatment, the seeds were rinsed with distilled water for 30 minutes. The seeds from each replicate were then placed on a moist filter paper in 9 cm Petri dishes at 25 °C. The number of seeds with emerging radicle or plumule was recorded after four days' incubation. A trait called *seed germination* (Table 6.1) was calculated by substracting the germination rate in waterlogging conditions from that in the control, then divided by germination rate in the control. Seeds used in this experiment have already had stored a long time after harvesting to overcome the dormancy problem.

Fig.6.1 The trials for phenotyping the doubled haploid populations were carried out in an incubator (left) and glasshouse (right).

Glasshouse trial

Four replicates of ten seeds for each DH and parental lines were sown in soil in 3.5 L pots (one pot per replicate) filled with soil from a frequently waterlogged site (Cressy Research Station) in Tasmania. After germination, five plants were kept in each pot and grown in a glasshouse, with natural daylength but temperature controlled to less than 24°C. Waterlogging treatments were conducted in children's paddling pools (Fig. 6.1). Each replicate was put into a different pool and the two populations were placed in pools of different size. A randomised design was used for each pool. Three replicates were subjected to waterlogging treatment and one replicate was grown under no-waterlogged conditions as a control in all the experiments. Waterlogging was started at the 3-leaf

stage, and lasted three to eight weeks depending on the trait measured.

The first trait measured was the proportion of each leaf which had lost its green colour (was yellow), this trait was called *leaf yellowing proportion*. Leaf yellowing proportion was chosen as the main indicator for waterlogging tolerance because other studies have found it to be correlated with yield reduction resulted from waterlogging stress (Van 1992). This trait was measured three times for each population. In Franklin/TX9425 population, the first recording was made in 2004 after two weeks waterlogging, and the second recording after four weeks treatment in the same experiment. The third measurement of leaf yellowing proportion in this population was taken after two weeks of waterlogging when the experiment was repeated in 2005. In population Franklin/Yerong, the first recording of leaf yellowing proportion was made in 2004 after two weeks waterlogging, whereas the second and third recordings were made in the repeated experiment carried out in 2005 after two weeks and four weeks of waterlogging stress, respectively. In both the populations, the plants in the control were growing well with no yellow leaf when this trait was measured in the waterlogging treatment. The proportion of leaf yellowing was estimated for each leaf. The length of each leaf was measured to weight the overall average proportion of yellow leaf in each plant. Then an average was calculated for all the plants in each pot.

The second trait measured was *plant biomass reduction*. This trait was measured in 2004 for DH lines from Franklin/Yerong population and in 2005 for DH lines from Franklin/TX9425 population. After three weeks waterlogging treatments, barley plants were cut at ground level and dried at 60 ^{o}C for four days in an electric oven. The average plant dry weight was measured for each replicate in control and in waterlogging treatments. Plant biomass reduction was calculated by subtracting the average dry weight of plants grown in waterlogging conditions from that in the control, then dividing by the average dry weight in the control.

The third measured trait was *plant survival*. After eight weeks of waterlogging, dead plants in each pot were counted after the water was drained. The measurement was carried out in 2004 for Franklin/TX9425 DH lines and in 2005 for Franklin/Yerong population. Plant survival was calculated as the numbers of surviving plants divided by the initial number of plants in each pot. All the traits measured in this study are listed in

Table 6.1.

Table 6.1 Traits measured in the two mapping populations.

Names of traits	Time when the traits were measured		Waterlogging stress	Brief names for QTL
	2004	2005		
Franklin/TX9425				
Leaf yellowing proportion 1.1	×		two weeks	tfy1.1
Leaf yellowing proportion 1.2	×		four weeks	tfy1.2
Leaf yellowing proportion 2.1		×	two weeks	tfy2.1
Plant survival	×		eight weeks	tfsur
Plant biomass reduction		×	three weeks	tfbio
Seed germination		×	six days	tfgerm
Franklin/Yerong				
Leaf yellowing proportion 1.1	×		two weeks	yfy1.1
Leaf yellowing proportion 2.1		×	two weeks	yfy2.1
Leaf yellowing proportion 2.2		×	four weeks	yfy2.2
Plant survival		×	eight weeks	yfsur
Plant biomass reduction	×		three weeks	yfbio
Seed germination		×	six days	yfgerm

6.2.1.3 Statistical analysis of phenotypic data

Statistical analysis was undertaken to detect significance of genetic effects for each trait in each population and also to calculate broad-sense heritability. For each experiment, the following mixed-effects model was used: $Y_{ij} = \mu + r_i + g_j + w_{jl.}$ Where: Y_{il} = observation on the *j*th genotype planted in the *i*th replication; μ = general mean; r_i = effect due to *i*th replication; g_j = effect due to the *j*th progeny; w_{ij} = error or genotype by replication interaction, where genotype was random and replicate treated as a fixed effect in analysis conducted using PROC MIXED of SAS. As part of the model checking procedure, SAS PROC UNIVARIATE was used to verify that the residuals were normally distributed. The broad-sense heritability was calculated as the ratio of the genetic variation (genotype) divided by phenotypic variation (due to genotype and residual). In order to calculate least square means for each genotype by trait by population by experiment combinations, PROC GLM was used with the same model as above, except that genotype was treated as a fixed effect. The normality of the least square means was checked using

SAS PROC UNIVARIATE for skewness and kurtosis.

6.2.2 QTL analysis

6.2.2.1 QTL detection by one-dimensional analysis

Using the software package MapQTL5.0 (Van Ooijen, 2004), quantitative traits were first analysed by interval mapping (IM), followed by composite interval mapping (CIM). The closest marker at each putative QTL identified using interval mapping was selected as a cofactor and the selected markers were used as genetic background controls in the approximate multiple QTL model (MQM) of MapQTL5.0. Log of the odds (LOD) threshold values applied to declare the presence of a QTL were estimated by performing the permutation tests implemented in MapQTL version 5.0 using at least 1000 permutations of the original data set for each trait, resulting in a 95% LOD threshold between 2.7 and 3.0.

To obtain 95% confidence intervals around the estimated point, a two-LOD support interval was established, by taking the two positions, left and right of the peak, that had a LOD value of two less than the maximum (Van Ooijen, 2004). The estimated additive genetic effect and the percentage of variance explained by each QTL and the total variance explained by all the QTLs affecting a trait, were obtained using restricted MQM mapping implemented with MapQTL5.0.

6.2.2.2 Analysis of QTL by digenic epistatic effects and QE interaction effects

In each of the two populations, leaf yellowing proportion measured in 2004 and 2005 after two weeks of waterlogging was used in this analysis. The DH lines in the 2004 experiments were planted in spring (mid November 2004) and the experiment terminated by the end of January 2005, while the 2005 experiments started in early May and ended by mid-July. The difference in planting time (season) provided different environmental conditions such as temperature, and day length between the two experiments.

A mixed-model-based composite interval mapping method (MCIM) was used for analysis of QTL by digenic epistatic effects and QE interaction effects implemented with QTLNetwork 2.0 (Yang et al. 2005). This included estimating and mapping main effect QTLs for additive effects at individual loci, interaction between two different loci, and

interactions between QTLs and environments. For ease of description, the QTLs with main effects that corresponded to QTLs detected by single-locus or interval analysis will be referred to as main-effect QTLs, and QTLs involved in digenic interactions as epistatic QTLs. Because DH populations that were homologous at all loci were used in this study, additive and dominance effect could not be separated.

In the analysis, the likelihood ratio (LR) and the t-test were combined to test the hypothesis on both genetic effects (including single locus effects and digenic epistatic effects) and QE effects. Estimates of QTL main effects were obtained by the maximum-likelihood estimation method, while QE effects were predicted by the best-linear-unbiased prediction (BLUP) method, with the environmental effect regarded as random. Thus the significance test for the predicted QE effects by BLUP has lower power. As a remedy, a Bayesian test was used for the significance test. In this study, the LR value corresponding to P = 0.005 was used as the threshold for claiming the presence of putative main or epistatic QTLs as recommended by Xing et al. (2002). The peak points of the LR and the t-test statistics in the linkage map were taken as the putative positions of the QTLs. When a QTL was involved in more than one epistasis, its position and effect were presented as the arithmetic mean of the values obtained from the different calculations. The relative contribution of a genetic component was calculated as the proportion of phenotypic variance explained by that component.

6.3 Results and analysis

6.3.1 Quantitative trait values in two barley DH populations

6.3.1.1 Phenotypic and genetic variation among the DH lines of the two populations

Leaf yellowing proportion, plant survival and plant biomass reduction following waterlogging stress showed normal distributions for both populations (Fig. 6.2). Summary statistics for each trait are presented in Table 6.2 for the Franklin/TX9425 population and Table 6.3 for the Franklin/Yerong population. Transgression beyond the parental values was observed for all traits including those for which parental values hardly differed. Variance estimates for the components are presented in Table 6.4 and 6.5. There was significant variation between DH lines (genetic variation) in each

population for all the measured traits. The effect of replication was not significant for traits measured early in the experiments, but was significant for most traits measured later. For all the traits analyzed, significant variation was observed between the DH lines within population as indicated by the broad sense heritabilities ranging from 0.71 to 0.11 in the Franklin/TX9425 population (Table 6.4) and ranging from 0.57 to 0.20 in of the Franklin/Yerong population (Table 6.5), respectively. This amount of genetic variation indicated that QTL mapping was likely to reveal QTLs for most of the traits.

a. Seed germination of the Franklin/TX9425 population

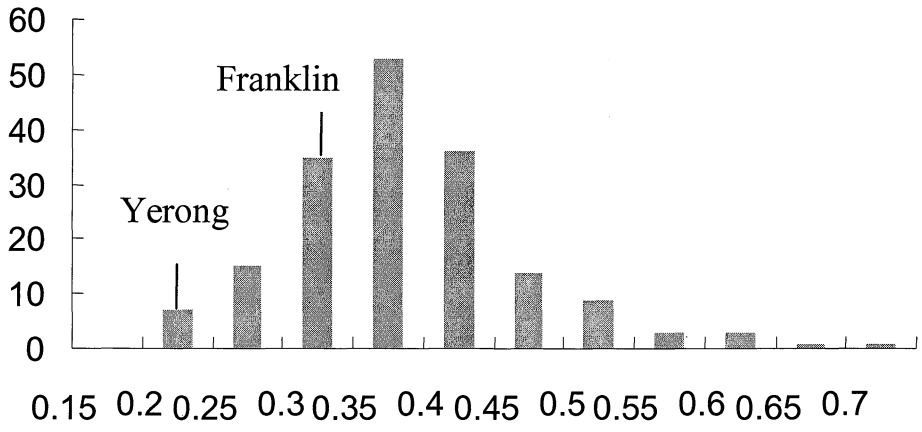
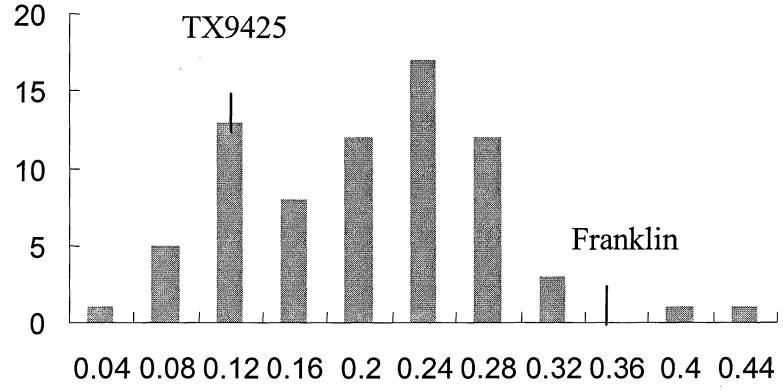
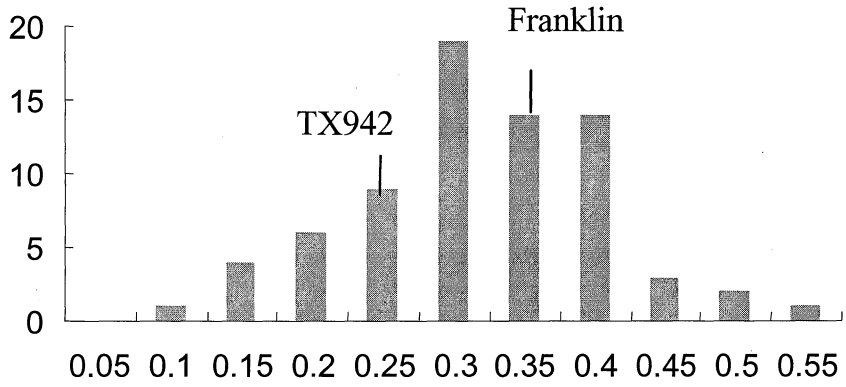


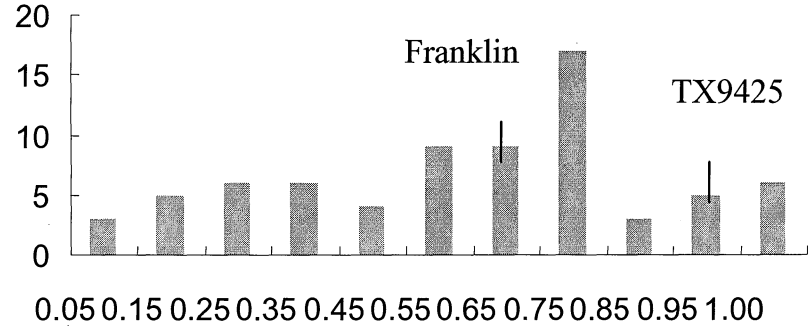
Fig.6.2 Frequency distribution of the waterlogging tolerance related traits measured in the two mapping populations. For each graph, the X axis represents the trait values, while the Y axis represents the number of DH lines whose mean values were between the related figures on the X axis. The graphs were named serially by a, b, c, d, e, f, g, h, i, j, and k. Figures in the definition of some traits denote the experimental conditions i.e. 1.1 = 2 weeks stress in 2004; 1.2 = 4 weeks stress in 2004; 2.1 = 2 weeks stress in 2005; 2.2 = 4 weeks stress in 2005.



b. Leaf yellowing proportion 1.1 of Franklin/TX9425 population

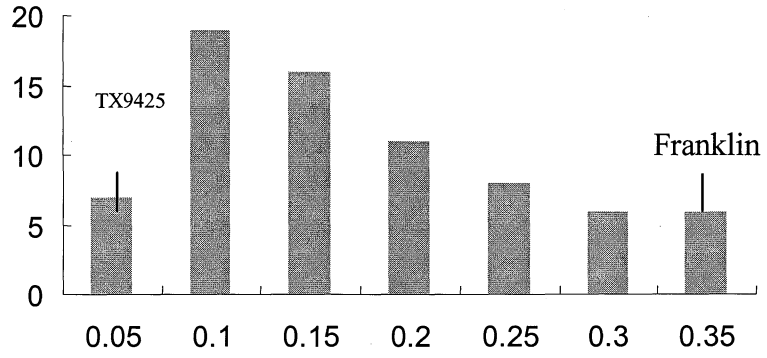


c. Leaf yellowing proportion 1.2 of Franklin/TX9425 population

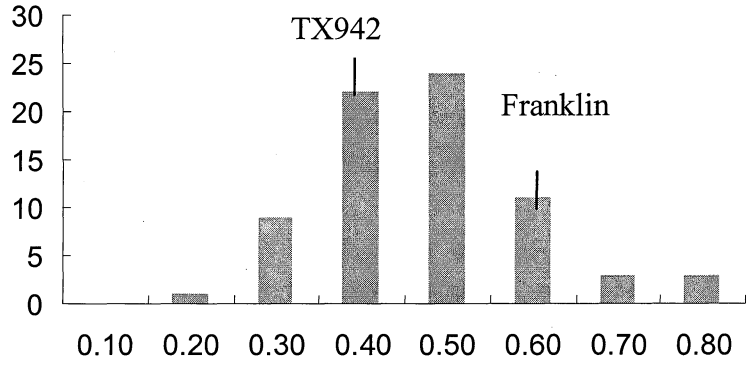


d. Plant survival of Franklin/TX9425 population

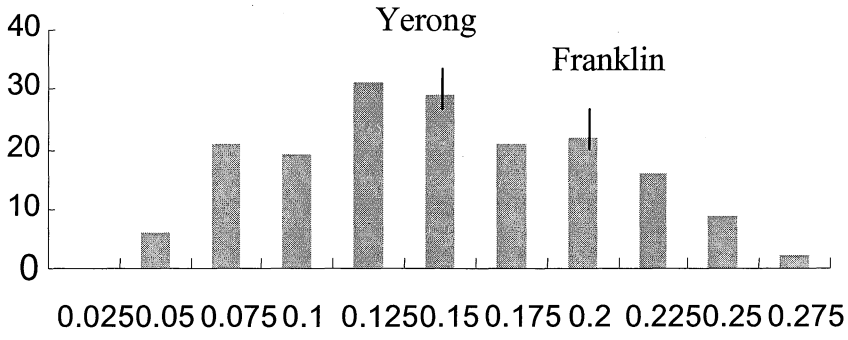
Fig. 6.2 Continued



e. Leaf yellowing proportion 2.1of Franklin/TX9425 population

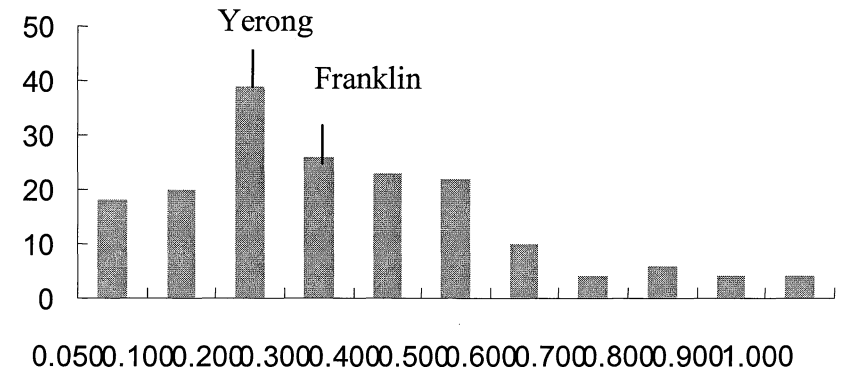


f. Plant biomass reduction of Franklin/TX9425 population

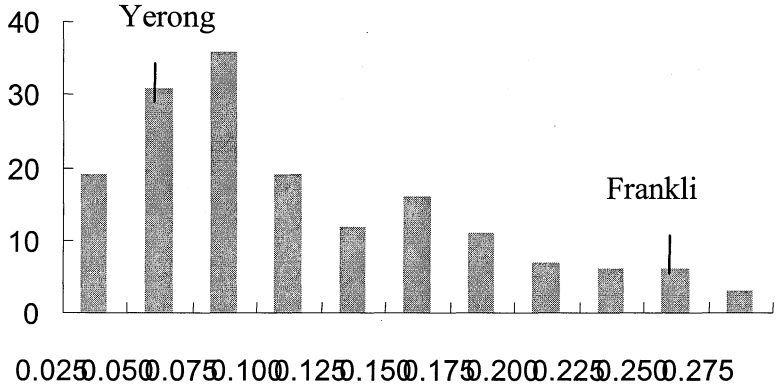


g. Leaf yellowing proportion 1.1 of Franklin/Yerong population

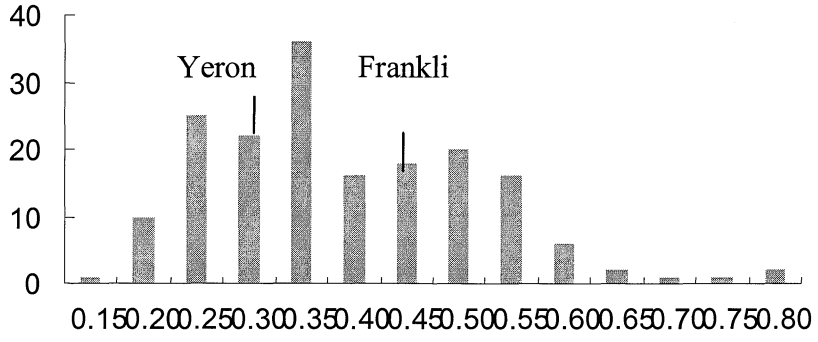
Fig. 6.2 Continued



h. Plant survival of Franklin/Yerong population

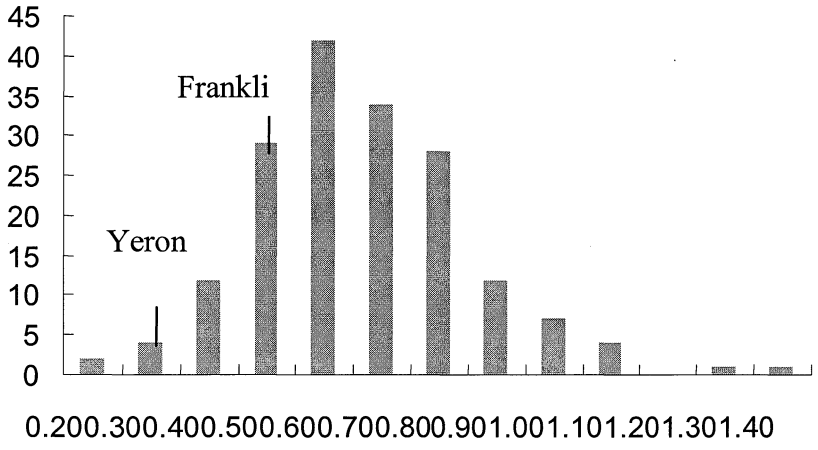


i. Leaf yellowing proportion 2.1 of Franklin/Yerong population



j. Leaf yellowing proportion 2.2 of Franklin/Yerong population

Fig. 6.2 Continued



k. Plant biomass reduction of Franklin/Yerong population

Fig 6.2 Continued

Table 6.2 Descriptive statistics of the investigated traits in the Franklin/TX9425 population, with means of each parent, and minimum/maximum/mean values of DH lines and standard deviation (SD).

	Mean for parents		DH lines			
Traits	TX9425	Franklin	Minimum	Maximum	Mean	SD
Leaf yellowing proportion1.1	0.1	0.34	0.04	0.4	0.19	0.08
Leaf yellowing proportion1.2	0.21	0.34	0.1	0.54	0.3	0.09
Plant survival	0.93	0.74	0	1	0.55	0.28
Leaf yellowing proportion2.1	0.05	0.34	0.02	0.35	0.16	0.09
Plant biomass reduction	0.37	0.51	0.18	0.71	0.43	0.11

Table 6.3 Descriptive statistics of the investigated traits in the Franklin/Yerong population, with means of each parent, and minimum/maximum/mean values of DH lines and standard deviation.

	Mean for parents		DH lines			
Traits	Yerong	Franklin	Minimum	Maximum	Mean	SD
leaf yellowing proportion1.1	0.13	0.19	0.04	0.27	0.14	0.05
Plant biomass reduction	0.28	0.44	-0.05	1.05	0.39	0.19
Leaf yellowing proportion 2.1	0.05	0.24	0	0.27	0.09	0.06
Leaf yellowing proportion 2.2	0.28	0.38	0.15	0.65	0.34	0.08
Plant survival	0.22	0.2	0	1	0.3	0.23

Table 6.4 Variance component estimates and broad-sense heritability (H^2) for traits measured in waterlogging experiment in the populations of Franklin/TX9425 DH lines.

	Variance component (estimate)			DH line		Replication	
Variable	DH line	residual	H^2	Z	Prob Z	F	Prob F
Leaf yellowing proportion1.1	0.005	0.004	0.563	4.72	<.0001	0.51	0.6026
Leaf yellowing proportion1.2	0.001	0.009	0.105	3.48	0.0003	4.06	0.0193
Plant survival	0.044	0.1	0.306	3.27	0.0005	24.04	<.0001
Leaf yellowing proportion 2.1	0.006	0.003	0.712	4.92	<.0001	5.11	0.0268
Plant biomass reduction	0.006	0.014	0.299	2.43	0.0075	17.03	<.0001

Table 6.5 Variance component estimates and broad-sense heritability (H^2) for traits measured in waterlogging experiment in the populations of Franklin/Yerong DH lines.

	Variance component (estimate)			DH line		Replication	
Variable	DH line	residual	H^2	Z	Prob Z	F	Prob F
Leaf yellowing proportion1.1	0.002	0.004	0.336	5.43	<.0001	0.35	0.7082
Leaf yellowing proportion 2.1	0.003	0.012	0.198	3.69	<.0001	2.9	0.0565
Plant survival	0.027	0.081	0.251	4.43	<.0001	3.38	0.0352
Leaf yellowing proportion 2.2	0.003	0.002	0.574	6.58	<.0001	15.59	0.0001
Plant biomass reduction	0.013	0.048	0.216	2.79	0.003	31.89	<.0001

6.3.1.2 Phenotypic correlations between traits in each population

The Pearson correlation coefficients for correlations among the traits investigated in the greenhouse experiment are listed in Table 6.6 for the population of Franklin/TX9425 and Table 6.7 for population of Franklin/Yerong. Among the investigated traits, significant positive correlations were found between all the leaf yellowing measurement, including

that between the different years (experiments). Significant positive correlations were found between all leaf chlorosis measurements and biomass reduction in the Franklin/TX9425 population, but not in the Franklin/Yerong population. Plant survival was poorly correlated with leaf chlorosis and only one of the correlations was significant. Plant biomass reduction and survival were not correlated with each other.

Table 6.6 Correlations between trait values used in QTL analysis in Franklin/TX9425 population. 1.1 = 2 weeks waterlogging stress in 2004; 1.2 = 4 weeks stress in 2004; 2.1 = 2 weeks stress in 2005; *** = $p < 0.005$, ** = $p < 0.01$, * = $p < 0.05$; p = probability.

	Leaf yellowing proportion1.2	Plant survival	Leaf yellowing proportion2.1	Plant biomass reduction
Leaf yellowing proportion1.1	0.70***	-0.07	0.67***	0.47***
Leaf yellowing proportion1.2		-0.34**	0.51***	0.28*
Plant survival			0.06	0.20
Leaf yellowing proportion2.1				0.43***

Table 6.7 Correlations between trait values used in QTL analysis in the population of Franklin/Yerong. 1.1 = 2 weeks waterlogging stress in 2004; 2.1 = 2 weeks stress in 2005; 2.2 = 4 weeks stress in 2005; *** = $p < 0.005$, * = $p < 0.05$; p = probability.

	Leaf yellowing proportion 2.1	Leaf yellowing proportion 2.2	Plant survival	Plant biomass reduction
Leaf yellowing proportion 1.1	0.32***	0.29***	0.20	-0.02
Leaf yellowing proportion 2.1		0.78***	-0.04	0.15*
Leaf yellowing proportion 2.2			0.00	0.17*
Plant survival				-0.11

6.3.2 Identification of QTLs in the Franklin/TX9425 population

Seed germination

Only one QTL (*tfgerm*) for waterlogging tolerance of barley seeds at the germination stage was identified on chromosome 1H (Table 6.8; Fig.6.3). This QTL explained 18.4% of the genetic variation. The Franklin allele at this locus increased the germination reduction rate under waterlogging stress.

Leaf yellowing proportion

Four QTLs (*tfy1.1-1, tfy1.1-2, tfy1.1-3* and *tfy1.1-4*) controlling leaf yellowing after two-weeks waterlogging stress (2004) were identified (Table 6.8, Fig.6.3). The four QTLs explained 23.3%, 33.4%, 5.3% and 7.1% of the genetic variation of leaf chlorosis, respectively. A total of 69.1% of the variation for leaf chlorosis was explained by these QTLs. The QTL *tfy1.1-1* was located on chromosome 2H, both *tfy1.1-2* and *tfy1.1-3* were mapped to chromosome 3H, with a 65 cM distance between them, whereas *tfy1.1-4* was located on chromosome 1H. For all the detected QTLs, the Franklin alleles increased leaf chlorosis while the TX9425 alleles decreased it.

Table 6.8 Characteristics of the detected QTLs explaining waterlogging related traits in Franklin/TX9425 population.

Trait	QTL	Chr.	Map position	LOD score	% of variance	Parent allele
Seed germination under flooding	*tfgerm*	1H	30	3.13	18.4	Franklin
Leaf yellowing proportion 1.1	*tfy1.1-1*	2H	82-92	9.21	23.3	Franklin
(two weeks stress, 2004)	*tfy1.1-2*	3H	67-69	7.59	33.4	Franklin
	tfy1.1-3	3H	1	3.22	5.3	Franklin
	tfy1.1-4	1H	32-36	2.72	7.1	Franklin
Leaf yellowing proportion 1.2 (four weeks stress, 2004)	*tfy1.2-1*	3H	69	7.31	36	Franklin
Leaf yellowing proportion 2.1	*tfy2.1-1*	3H	69-74	9.28	34.1	Franklin
(two weeks stress, 2005)	*tfy2.1-2*	7H	78-104	3.62	16	Franklin
Plant survival	*tfsur-1*	2H	49-67	3.29	19	Franklin
	tfsur-2	2H	95	2.7	13.2	TX9425
Plant biomass reduction	*tfmas*	4H	45-49	2.75	16.3	Franklin

One QTL (*tfy1.2-1*) was identified for leaf yellowing rate after four-weeks waterlogging (2004) treatment. It is mapped on chromosome 3H, explaining 36% of the genetic variation. The Franklin allele increased the yellowing leaf proportion. This is likely to be the same QTL as tfy1.1-2 as both located at a similar position on chromosome 3H and because it basically is a repeated measurement.

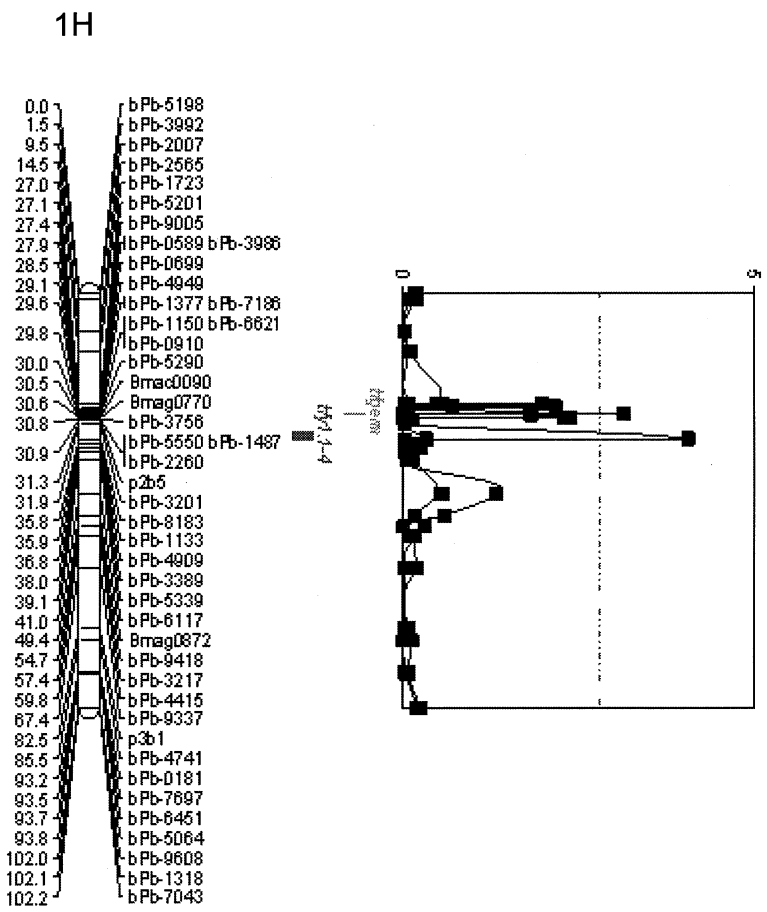


Fig.6.3 The Franklin/TX9425 linkage map showing the locations of QTLs for the traits analyzed. Each linkage group consists of a vertical bar on which the map positions and names of loci are indicated. On the right, QTL intervals and QTL LOD graphs are shown. QTL intervals are specified by their start and end point, for each QTL, and an inner and an outer interval was specified. QTL graphs exported from MapChart software were specified by a reference to a text file containing the map positions and functional values (e.g., LOD values) of graph points. The Y axis of the graph corresponds to the linkage map with LOD scores on X axis.

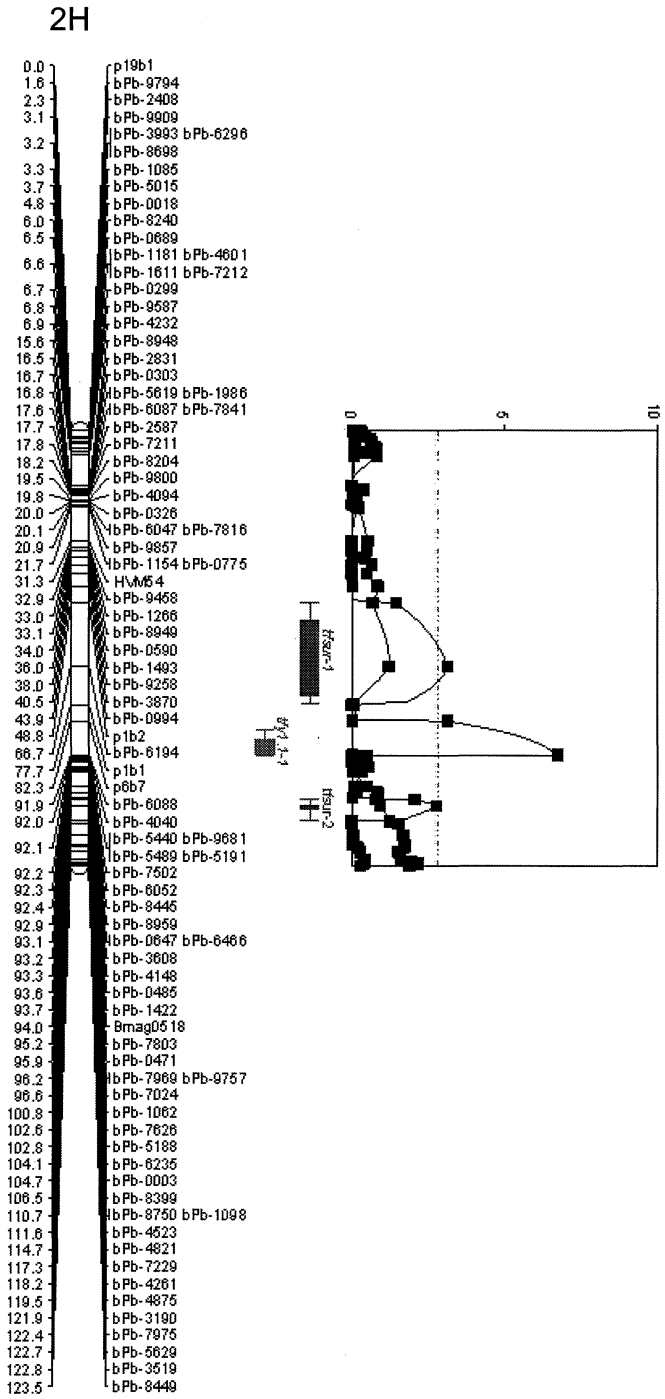


Fig. 6.3 Continued

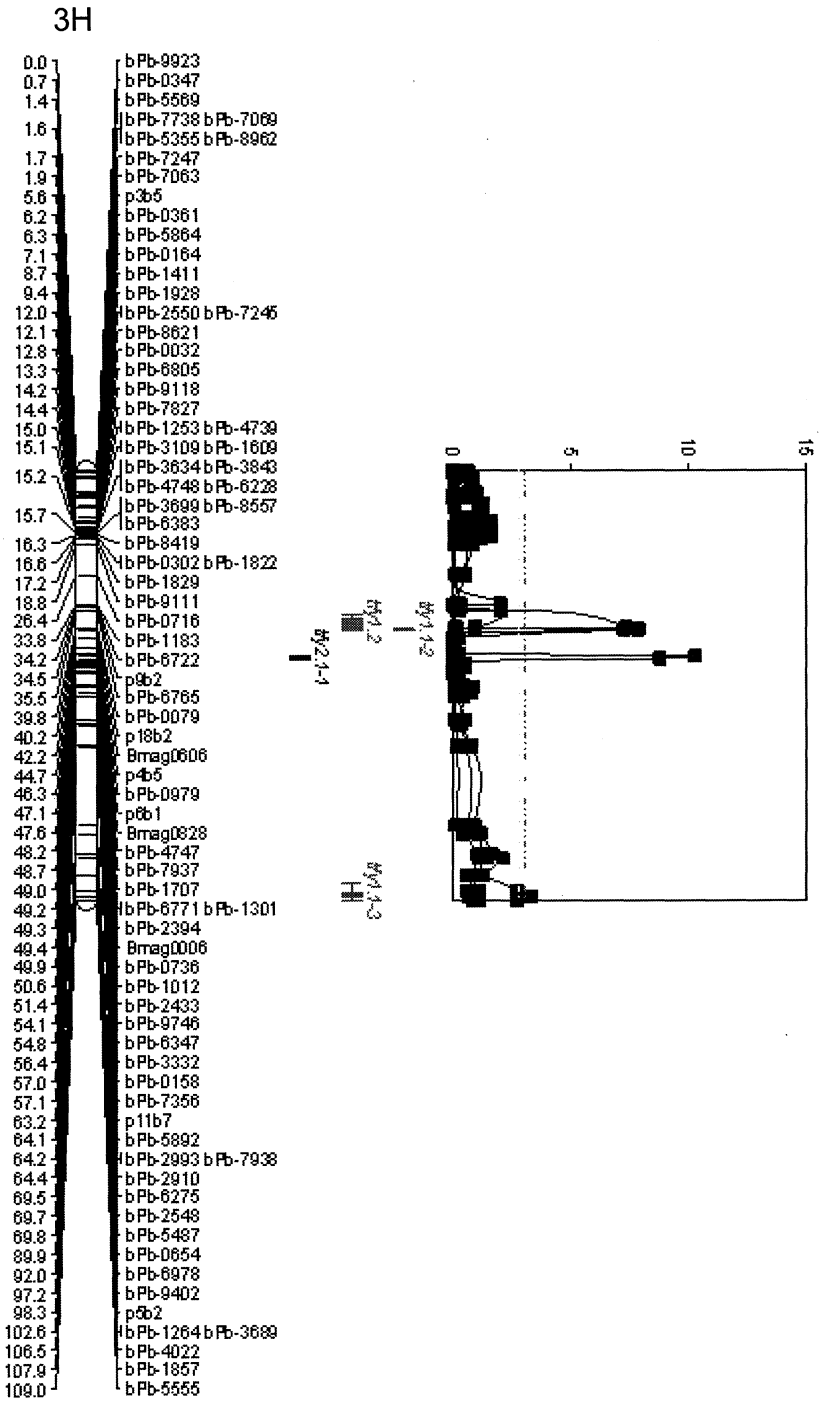


Fig. 6.3 Continued

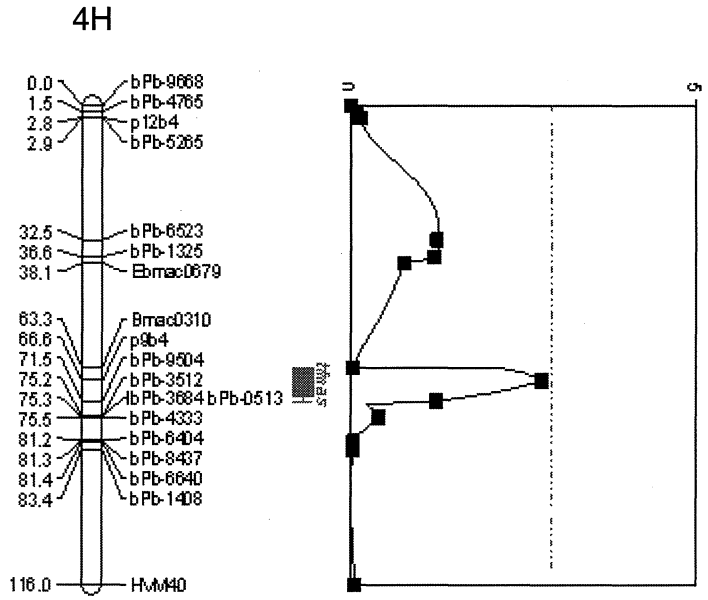


Fig. 6.3 Continued

Two QTLs (*tfy2.1-1 and tfy2.1-2*) were found for leaf yellowing rate in the experiment carried out in 2005. QTL *tfy2.1-1* was located on chromosome 3H and another one (*tfy2.1-2*) was mapped on chromosome 7H. These QTLs explained a total of 50.1% of the genetic variation of leaf yellowing rate, with an individual effect of explaining 34.1%, and 16% of the genetic variation, respectively. The Franklin allele increased the yellowing leaf proportion at both loci. QTL *tfy2.1-1* is likely to be the same as *tfy1.1-2* and *tfy1.2-1*.

Plant survival

Two QTLs (*tfsur-1* and *tfsur-2*) were found for plant survival rate after eight weeks continuous waterlogging stress. Both of them were located on chromosome 2H, explaining 19% and 13.2% of the genetic variation for this trait, respectively. These QTL were located onto different regions of chromosome 2H compare with the QTLs for leaf chlorosis. This confirms the statistical analysis results of no significant correlation between these two traits (Table 6.6). For the detected QTLs, the Franklin allele increased the survival rate of the plant at *tfsur-1* locus, whereas TX9425 allele increased plant

survival at the locus of *tfsur-2*. This may explain the strong transgressive segregation found for this trait.

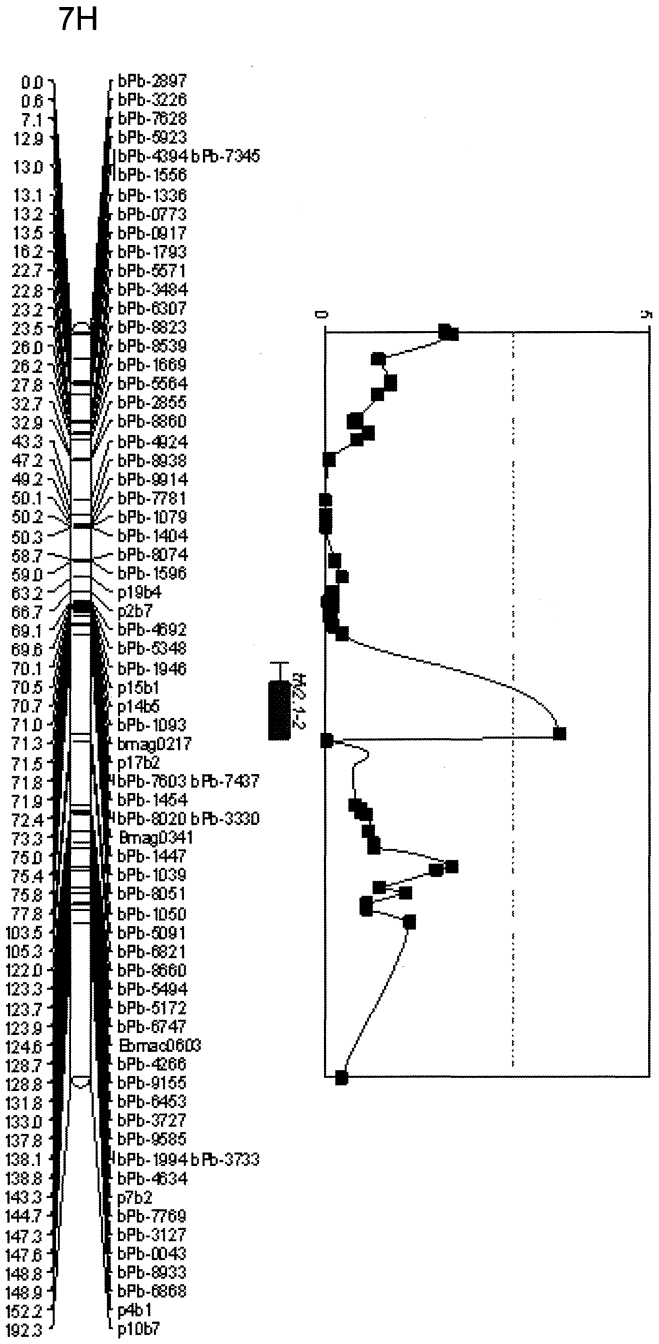


Fig. 6.3 Continued

Reduction of plant biomass

Although the difference in the reduction of plant biomass due to waterlogging stress between TX9425 and Franklin was small, one QTL (*tfmas*) was identified for plant dry weight reduction after two-weeks waterlogging stress. This QTL was mapped to chromosome 4H and explained 16.3% of the genetic variation of this trait. Compared to the TX9425 allele, the Franklin allele increased reduction of plant biomass.

6.3.3 Identification of QTLs in the population of Franklin/Yerong

Leaf yellowing proportion

Three QTLs (*yfy1.1-1, yfy1.1-2 and yfy1.1-3*) controlling leaf yellowing after two-weeks waterlogging stress (2004) were found on chromosome 3H, 2H and 5H, explaining 10%, 8.9% and 8.6% of the genetic variation, respectively. For the detected QTLs, the Franklin alleles increased the yellowing rate at two QTLs (*yfy1.1-1 and yfy1.1-2*), whereas at the *yfy1.1-3* locus the Yerong allele increased leaf yellowing proportion (Table 6.9, Fig.6.4).

Table 6.9 Characteristics of the detected QTLs explaining waterlogging related traits in Franklin/Yerong population.

Trait	QTL	Chr.	Map position (cM)	LOD score	% of variance	Parental allele
Leaf yellowing proportion 1.1 (two weeks stress, 2004)	*yfy1.1-1*	3H	28-31	2.74	10	Franklin
	yfy1.1-2	2H2	46-55	3.96	8.9	Franklin
	yfy1.1-3	5H	59	3.97	8.6	Yerong
Leaf yellowing proportion 2.1 (two weeks stress, 2005)	*yfy2.1-1*	7H	93-99	3.72	6.7	Franklin
	yfy2.1-2	3H	33-36	6.41	11.9	Franklin
	yfy2.1-1	4H	26-36	9.25	18.5	Franklin
Leaf yellowing proportion 2.2 (four weeks stress, 2005)	*yfy2.2-1*	3H	33-36	5.03	9.5	Franklin
	yfy2.2-2	1H	54	2.77	5	Yerong
	yfy2.2-3	4H	26-36	10.37	22.4	Franklin
Plant survival	*yfsur-1*	2H2	44-46	3.15	7.1	Franklin
	yfsur-2	5H	46-58	5.05	13.1	Yerong
Reduction of plant biomass	*yfbio*	4H	25-31	3.03	8.2	Franklin

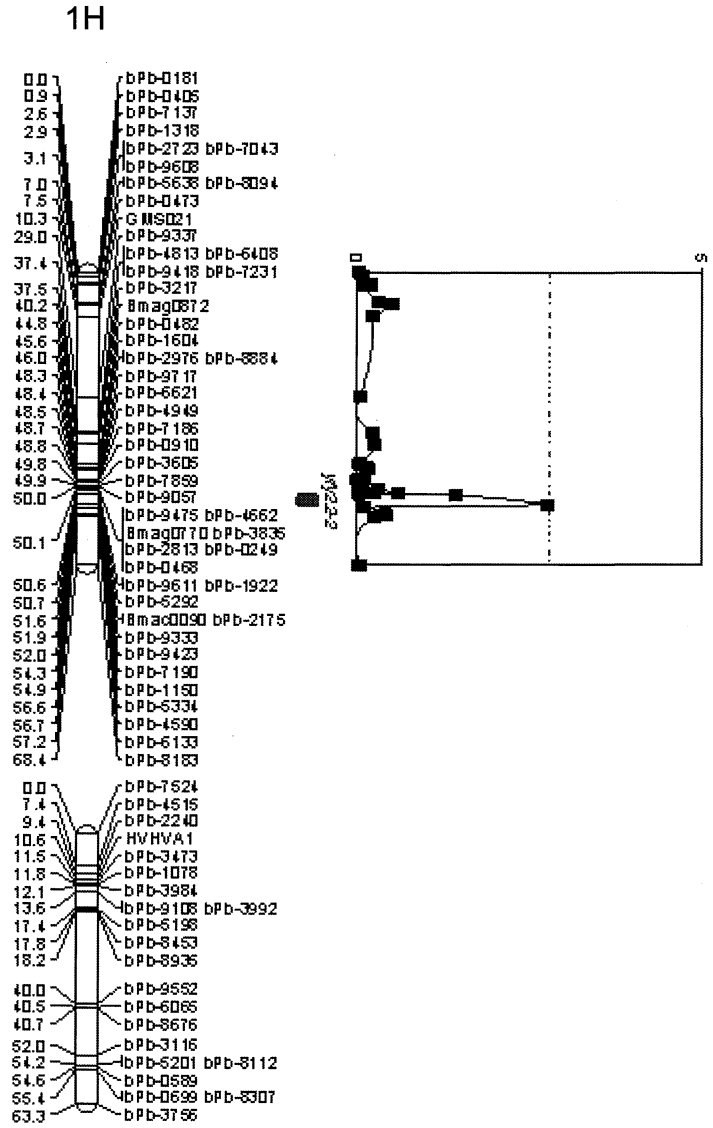


Fig.6.4 The Franklin/Yerong linkage map showing the locations of QTLs for the traits analyzed. Each linkage group consists of a vertical bar on which the map positions and names of loci are indicated. On the right, QTL intervals and QTL LOD graphs are shown. QTL intervals are specified by their start and end point, for each QTL, and inner and an outer interval was specified. QTL graphs exported from MapChart software were specified by a reference to a text file containing the map positions and functional values (e.g., LOD values) of graph points. The Y axis of the graph corresponds to the linkage map and LOD scores on X axis.

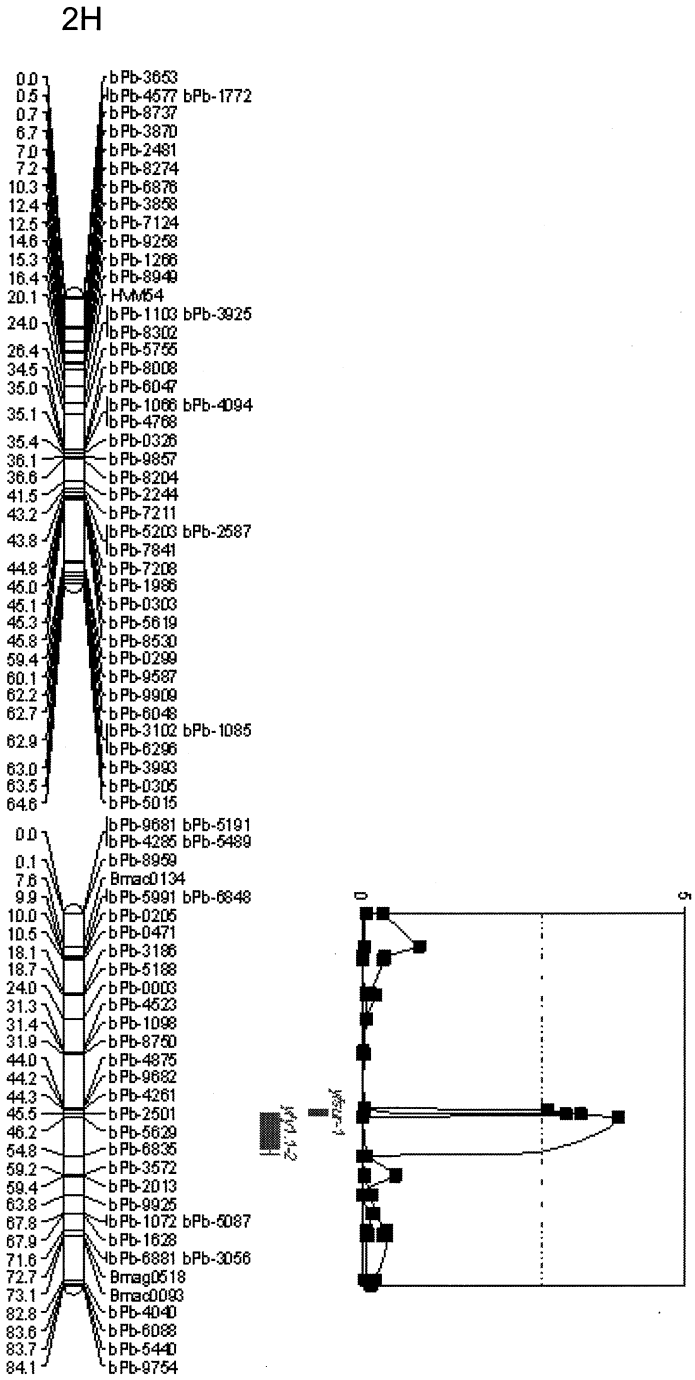


Fig. 6.4 Continued

Three QTLs (*yfy2.1-1, yfy2.1-2 and yfy2.1-3*) were found for leaf yellowing rate after two weeks of waterlogging in the experiment carried out in 2005, these QTLs were located on chromosome 7H, 3H and 4H, explaining 6.7%, 11.9% and 18.5% of the genetic variation, respectively. The Franklin alleles increased the leaf yellowing proportion in all three

cases. QTL *yfy2.1-2* may be the same as *yfy1.1-1* as they located at a similar position on chromosome 3H.

Three QTLs (*yfy2.2-1, yfy2.2-2 and yfy2.2-3*) were found for leaf yellowing rate after four weeks waterlogging stress in the experiment carried out in 2005, these QTLs were located on chromosome 3H, 1H and 4H, explaining 9.5%, 5.0% and 22.4% of the genetic variation, respectively. The Franklin allele increased leaf yellowing proportion at *yf2.2-1* and *yf2.2-3* loci, whereas the Yerong allele did so at the locus of *yf2.2-2*. QTL *yfy2.2-1* is likely to be the same as *yfy2.1-2* as it is in an identical position on chromosome 3H. The same applies to QTL *yfy2.1-1* and *yfy2.2-3* on chromosome 4H.

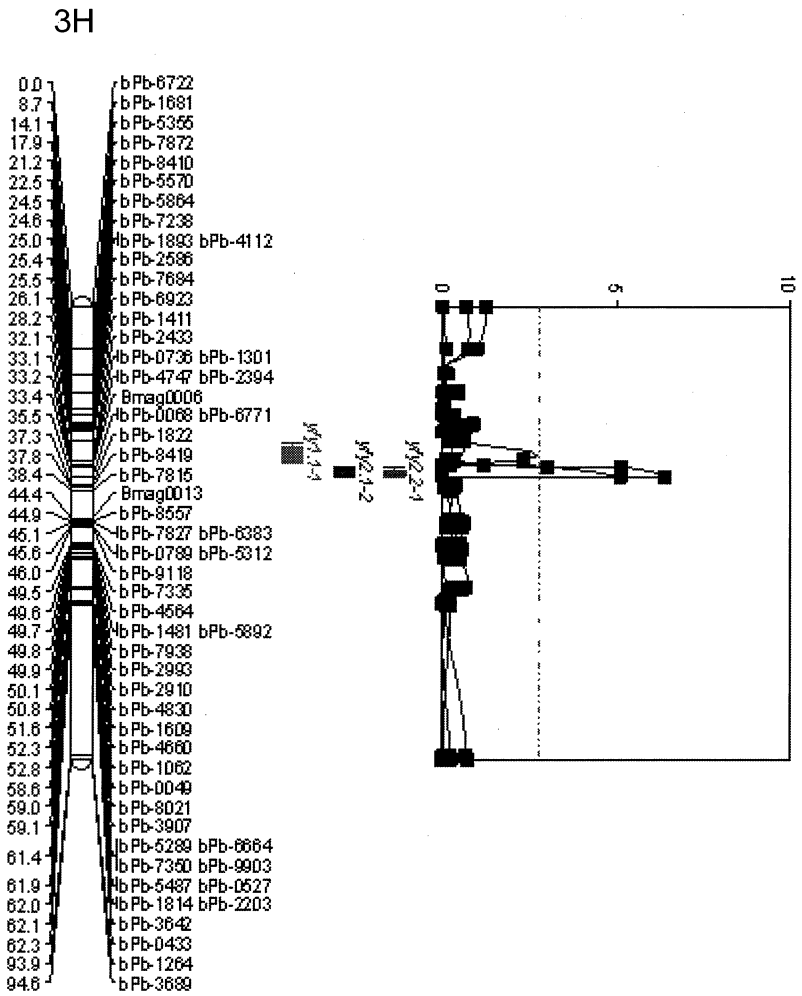


Fig. 6.4 Continued

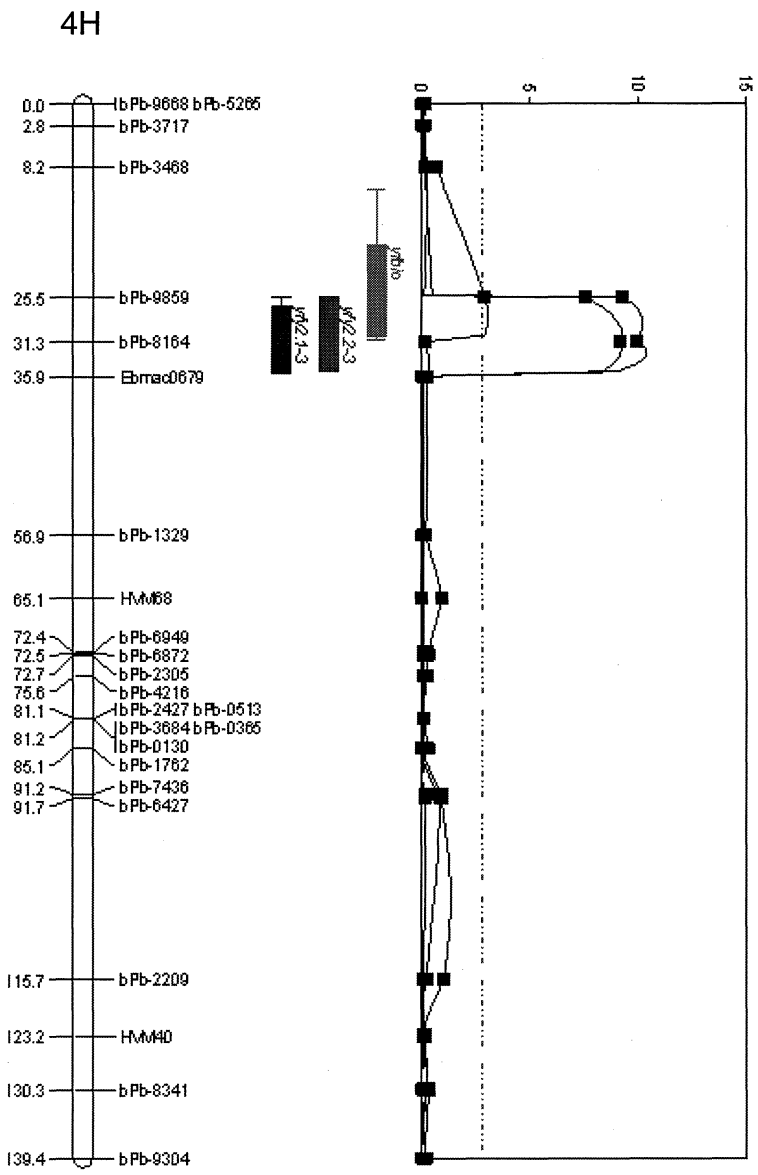


Fig. 6.4 Continued

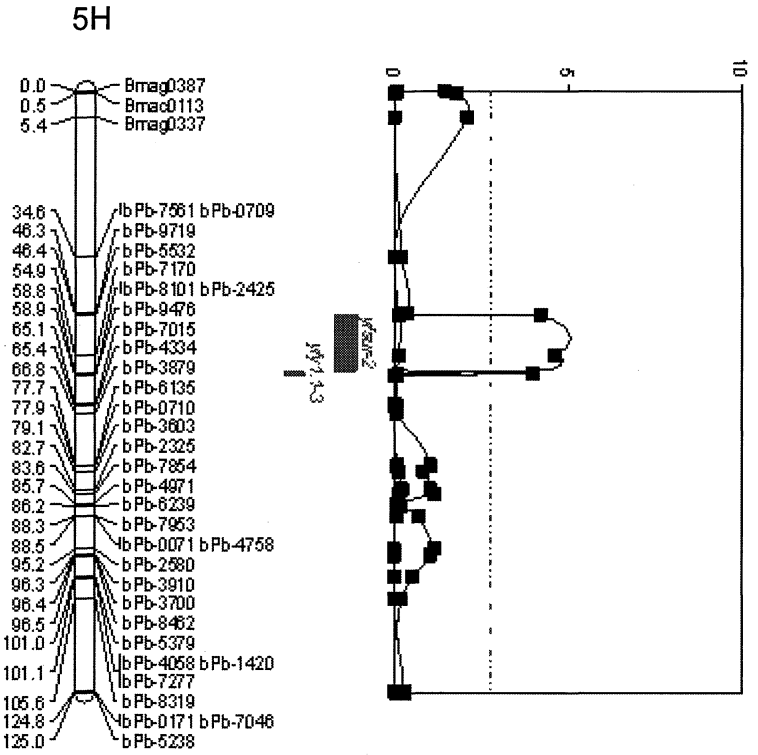


Fig. 6.4 Continued

Plant survival rate

Two QTLs (*yfsur-1 and yfsur-2*) were identified for plant survival rate after 8 weeks continuous waterlogging stress. They were located on chromosome 2H and 5H, explaining 7.1% and 13.1% of the genetic variation for this trait, respectively. The Franklin allele increased plant survival rate at the *yfsur-1* locus, and the Yerong allele increased plant survival rate at the *yfsur-2* locus.

Reduction of plant biomass

Although the difference in the reduction of plant biomass between Yerong and Franklin was small after three weeks of waterlogging stress, one QTL (*yfmas*) was identified. This QTL was mapped on chromosome 4H, and it explained 16.3% of the genetic variation of this trait. It was mapped onto the same chromosome region with QTLs identified for leaf yellowing. This confirmed the statistical analysis results of significant correlation between these two traits (Table 6.7).

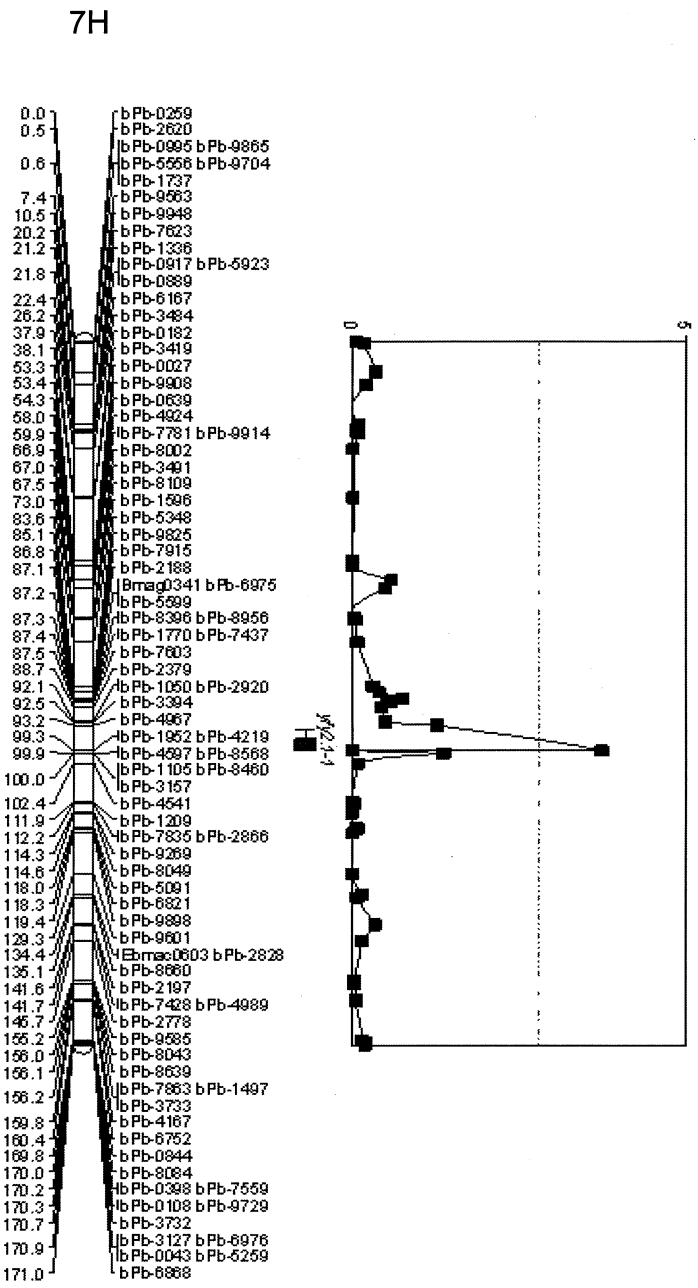


Fig. 6.4 Continued

6.3.4 Analysis of main effects, digenic epistatic effects and QE interaction effects for QTLs controlling leaf yellowing proportion.

In this study, for the purpose of estimating and mapping QTLs for main effects at an individual locus, interaction between two different loci, and interactions between QTLs and environments, the marker genotype data and trait phenotypic data were analysed

using a two-dimensional QTL analysis method (QTLNetwork 2.0) (Yang et al. 2005).

Four QTLs and one digenic epistatic interaction were identified for leaf yellowing proportion in the Franklin/TX9425 ($P<0.005$) population. The QTLs were named as "tf" along with the chromosomal number followed by the serial number. The genetic architecture information identified in this population was summarized to an informative QTL network map with the effect magnitudes being ignored (Fig. 6.5). These QTLs were located on chromosomes 1H, 2H, 3H and 7H (Table 6.10, Fig 6.5), respectively. The QTL located on chromosome 3H has comparatively higher effect (Table 6.10), this is in accordance with the one-dimensional analysis by MapQTL5.0. An epistatic interaction was identified between a locus on chromosome 3H and a locus on chromosome 7H (Table 6.11).

Table 6.10 Mapping results of single-locus effect QTLs significant at 0.005 level in Franklin/TX9425 population. QTLs are named with the relevant chromosome and the marker intervals. For example, the first QTL is named as 1-51, which means that this QTL was located at the 51st marker interval of the group 1. A: the estimated additive effect; AE1: the predicted additive by environment interaction effect in 2004; AE2: the predicted additive by environment interaction effect in 2005

QTL	Chr.	Interval	Position (cM)	A	AE1	AE2
tf1-51	2H	bPb-6088-bPb-4040	86-92	-0.013	-0.012	0.013
tf2-27	3H	bPb-2394-bPb-1301	60-61	-0.044	0.011	-0.011
tf3-50	7H	bPb-6821-bPb-8660	104-119	-0.026	0.009	-0.009
tf5-26	1H	bPb-8183-bPb-1133	34-39	-0.013	-0.005	0.005

Table 6.11 The two loci involved in epistatic interaction. AA: the estimated additive by additive effect; AAE1: the predicted aa by environment interaction effect in 2004 experiment; AAE2: the predicted aa by environment interaction effect in 2005 experiment.

Epistatic locus	Chr.	Interval	Position (cM)	AA	AAE1	AAE2
tf2-39	3H	bPb-0079-bPb-6765	68-73			
tf3-2	7H	bPb-3226-bPb-7628	0-7	0.023	0	0

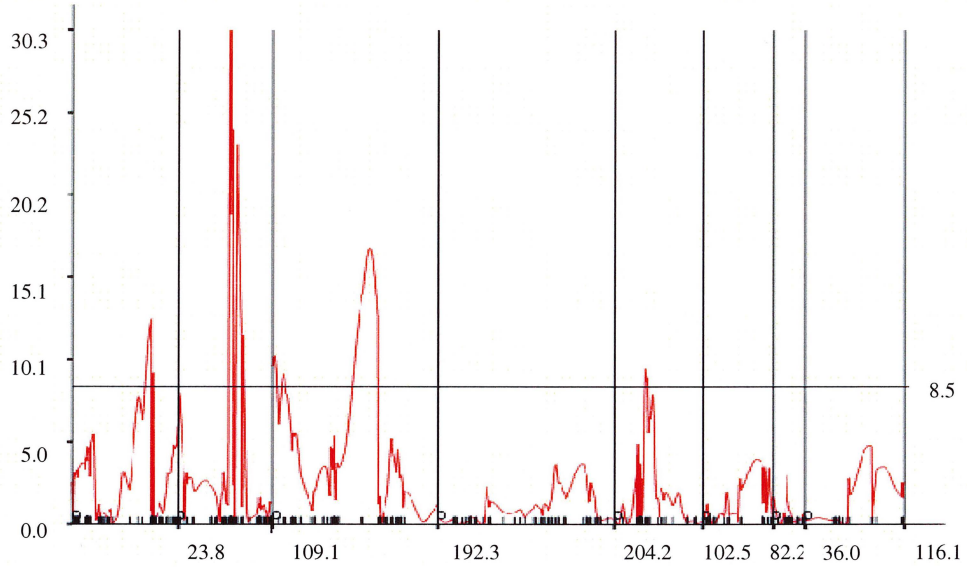


Fig.6.5 1D visualization of the test statistics of genome scan for QTL and epistasis in Franklin/TX9425 population. X axis is the different linkage groups starting with one on the left; Y axis is the F value for each detected QTL.

Six QTLs were identified for leaf yellowing proportion in the population of Franklin/Yerong using two-dimensional QTL analysis ($P < 0.005$) (Table 6.12, Fig 6.6). The QTLs were named as "yf" along with the chromosomal number followed by the serial number. The whole genetic architecture information identified in this population was summarized to an informative QTL network map with the effect magnitudes being ignored (Fig.6.6). These QTLs were located on chromosomes 2H1, 2H2, 3H, 4H, 5H and 7H. The QTLs located on chromosomes 3H and 4H have comparatively higher effect (Table 6.12). The results are in accordance with the one-dimensional analysis by MapQTL 5.0. No epistatic interaction was identified in this population.

Table 6.12 Mapping results of single-locus effect QTLs in Franklin/Yerong population. QTL is named with the relevant chromosome and the marker intervals. For example, the first QTL is named as 1-46, it means that this QTL was located at the 46th marker interval of group 1. A represents the estimated additive effect; AE1: the predicted additive by

environment interaction effect in 2004; AE2: the predicted additive by environment interaction effect in 2005

QTL	Chr.	Interval	Position (cM)	A	AE1	AE2
yf1-46	7H	bPb-1952-bPb-4219	98-112	0.011	-0.0045	0.0045
yf2-22	3H	bPb-6771-bPb-1822	33-37	0.017	-0.0042	0.0042
yf5-25	2H1	bPb-9857-bPb-8204	33-42	0.011	0.0001	-0.0001
yf6-21	2H2	bPb-5629-bPb-6835	40-55	0.006	0.0078	-0.0077
yf7-11	5H	bPb-9476-bPb-7015	55-63	-0.009	-0.0056	0.0055
yf8-6	4H	bPb-8164-Ebmac0679	26-35	0.020	-0.0072	0.0079

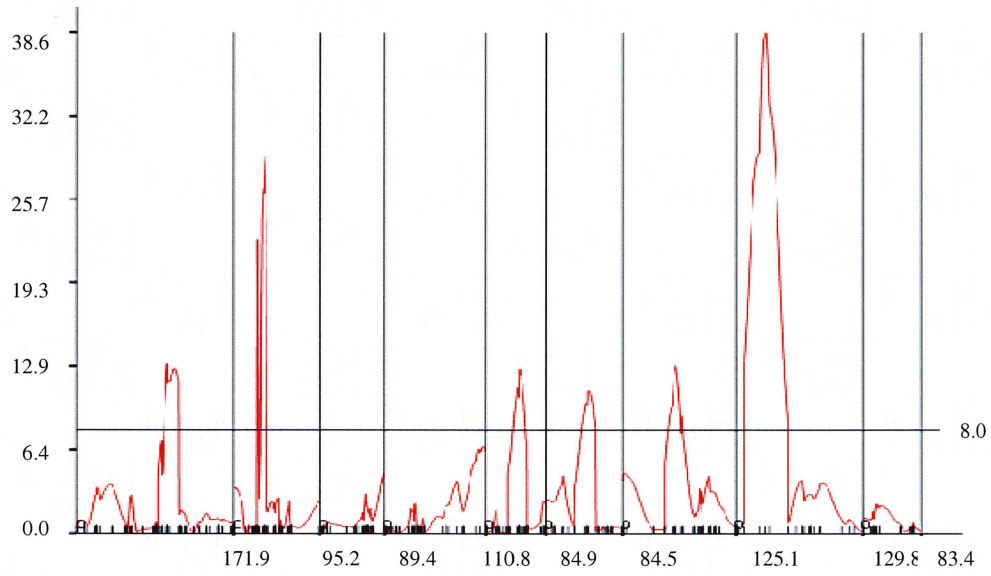


Fig.6.6 1D visualization for the test statistics of genome scan for QTL and epistasis in population of Franklin/Yerong. X axis is the different linkage groups starting with one on the left; Y axis is the F value for each detected QTL.

6.3.5 Comparison of QTLs associated with waterlogging tolerance in the two barley populations.

Table 6.13 shows that in the population of Franklin/Yerong, most of the QTLs located on

a specific chromosome were mapped to the same or a very similar region. For example, on chromosome 3H, QTL *yfy1.1-1, yfy2.1-1, yfy2.2-1* are the same QTL controlling leaf yellowing proportion. QTL *yfy2.1-1, yfy2.2-3* and *yfmas* were collocated on chromosome 4H. Similarly, a QTL controlling leaf yellowing proportion were collocated with a QTL controlling plant survival on both of the chromosomes 5H (*yfy1.1-3* and *yfsur-2*) and 2H (*yfy1.1-2* and *yfsur-1*). In Franklin/TX9425 population, some QTLs were also mapped to the same or a very similar region in each specific chromosome which has more than one QTL, For example, QTL *tfy1.1-2, tfy1.2-1* and *tfy2.1-1* were co-located on chromosome 3H, QTL *tfy1.1-4* and tfgerm were co-located on chromosome 1H, and QTL *tfy1.1-1* and *tfsur-2* were also mapped closely on chromosome 2H. QTL *tfsur-1*, however, seems to be a different locus to other QTLs identified on chromosome 2H.

Table 6.13 Comparison of QTLs identified in the two populations on a chromosome scale. The location in this table is based on each individual map.

Chromosome	Franklin/Yerong			Franklin/TX9425		
	QTLs	location (cM)	Effect (%)	QTLs	Location (cM)	Effect (%)
1H	*yfy2.2-2*	54	5	*tfy1.1-4*	32-36	7.1
				tfgerm	30	18.4
2H	*yfy1.1-2*	46-55	8.9	*tfy1.1-1*	82-92	23.3
	yfsur-1	44-46	7.1	*tfsur -1*	49-67	19.1
				tfsur-2	95	13.2
3H	*yfy1.1-1*	28-31	10	*tfy1.1-2*	67-69	33.4
	yfy2.1-2	33-36	11.9	*tfy1.1-3*	1	5.3
	yfy2.2-1	33-36	9.5	*tfy1.2-1*	68-69	36
				tfy2.1-1	69-74	34.1
4H	*yfy2.1-1*	26-36	18.6	*tfmas*	45-49	16.3
	yfy2.2-3	26-36	22.4			
	yfmas	25-31	8.2			
5H	*yfy1.1-3*	59	8.6			
	yfsur-2	46-58	13.2			
7H	*yfy2.1-1*	93-99	6.7	*tfy2.1-2*	78-104	16

In order to compare the QTLs identified in different populations or QTLs identified using different QTL analysis methods, the flanking markers of each QTL were relocated on the consensus map constructed using four different barley DH populations (Chapter 5). All the QTLs identified in this study are presented in Table 6.14. QTL tfy1.1-2, tfy2.1-1, yfy2.1-2 and yfy2.2-1, however, were not included in this table since, as explained earlier, they were likely to be the same as other QTLs.

Table 6.14 Comparison of QTLs identified in the two mapping population by using different QTL analysis methods. This table shows the position of each QTL in the consensus map.

Franklin/TX9425			Franklin/Yerong		
QTL	Chr.	interval	QTL	Chr.	interval
MapQTL 5.0			MapQTL 5.0		
tfgerm	1H	88.8-92.2	*yfy2.2-2*	1H	46.6-58.8
tfy1.1-4	1H	68.2	*yfy1.1-2*	2H	39.2-47.3
tfy1.1-1	2H	74.2-85.3	*yfsur-1*	2H	37.1-38.5
tfsur-1	2H	97.8	*yfy1.1-1*	3H	109.1-113.9
tfsur-2	2H	71.0-79.7	*yfy2.1-3*	4H	108.1-118.4
tfy1.1-3	3H	170.7	*yfbio*	4H	112.0-131.1
tfy1.2-1	3H	102.5	*yfy1.1-3*	5H	103.7-103.8
tfmas	4H	66.8	*yfsur-2*	5H	103.8-116.5
tfy2.1-2	7H	44.9-68.6	*yfy2.1-1*	7H	63.6-69.2
QTLNetwork2.0			QTLNetwork 2.0		
tf5-26	1H	65.0-68.2	*yf5-25*	2H	136.3-136.7
tf1-51	2H	74.2-74.7	*yf6-21*	2H	39.2-47.3
tf2-27	3H	114.4	*yf2-22*	3H	112.9
tf2-39	3H	93.4-102.5	*yf8-6*	4H	108.1-112.0
tf3-50	7H	31.0-44.4	*yf7-11*	5H	97.5-103.6
tf3-2	7H	132.5-136.8	*yf1-46*	7H	63.6-63.7

Table 6.14 shows that in the population of Franklin/TX9425, QTLs identified using MapQTL 5.0 and QTLNetwork 2.0 were quite similar. For example, *tf1-51* was the same QTL as *tfy1.1-1*; *tf2-39* same as *tfy1.2-1*. *tf3-50* same as *tfy2.1-2*; *tf5-26* same as *tfy1.1-4*; However, no counterparts were identified for *tf2-27* (located on chromosome 3H) and *tf3-2* (located on 7H), this may due to the one-dimentional analysis failing to detect the

epistatic interactions between these two loci. While in the population of Franklin/Yerong, *yf1-46* was the same QTL as *yfy2.1-1*; *yf2-22* co-located with *yfy1.1-1*; *yf6-21* was the same QTL as *yfy1.1-2*; *yf7-11* was the same QTL as *yfy1.1-3*; and *yf8-6* was the same QTL as *yfy2.1-3*. Among the QTLs identified in the Franklin/Yerong population, QTL *yf5-25* was a different locus from other QTLs located on chromosome 2H.

Table 6.15 Characterisation of QTLs or epistatic loci identified in this study, showing some SSR or RFLP markers closely linked to the QTLs based on a consensus map.

QTL	Consensus map		
	Chr.	interval	Markers closely linked
Franklin/TX9425			
tfgerm	1H	88.8-92.2	WG789B
tf5-26	1H	65.0-68.2	ABG464
tfsur-1	2H	97.8	EBmatc39
tfsur-2	2H	79.7	Bmag381
tf1-51	2H	74.2-74.7	bBE54D
tfy1.1-3	3H	170.7	ABG319B
tf2-27	3H	114.4	CDO113
tf2-39	3H	93.4-102.5	WG940; Myb
tfmas	4H	66.8	CDO795; ABC321; WG232
tf3-50	7H	31.0-44.4	ABC158; SSS1; KsuA1A
tf3-2	7H	132.5-136.8	MWG635B; EBmac755
Franklin/Yerong			
yfy2.2-2	1H	46.6-58.8	cMWG706A; ABC307A
yfsur-1	2H	37.1-38.5	MWG858
yf5-25	2H	136.3-136.7	BCD266; ABG317B
yf6-21	2H	39.2-47.3	MWG858; ABG358; ABG459
yf2-22	3H	112.9	CDO113
yfbio	4H	112.0-131.1	HvMLOH1;CDO63; HVM67
yf8-6	4H	108.1-112.0	Ebmac0679; WG114; iHxk2;
yfsur-2	5H	103.8-116.5	WG364; mSrh; KSUA1
yf7-11	5H	97.5-103.6	WG364
yf1-46	7H	63.6-63.7	MWG911

In order to compare the QTLs identified in different populations, the flanking markers of each QTL were relocated on the consensus map constructed using four barley DH

populations (in Chapter 5) including the two populations used in this study. Comparison of the identified QTLs between the two populations (Table 6. 13; Table 6.14; Figure 6.7) showed that many of the QTLs identified in Franklin/TX9425 mapped to similar chromosomal regions compared to those identified in Franklin/Yerong (such as QTLs identified on chromosome 1H, 3H, and 7H), or were mapped to a very close location (QTLs identified on chromosome 2H and 4H) with distances of less than 10 cM between QTL maxima (Figure 4). Some SSR and RFLP markers closely linked to the identified QTLs were selected from the consensus map and listed in Table 6.15. QTLs tfy1.1-4, tfy1.1-1, tfy1.2-1, tfy2.1-2; yfy1.1-2, yfy1.1-1, yfy2.1-3, and yfy1.1-3 were not included in this table because they are same as other QTLs.

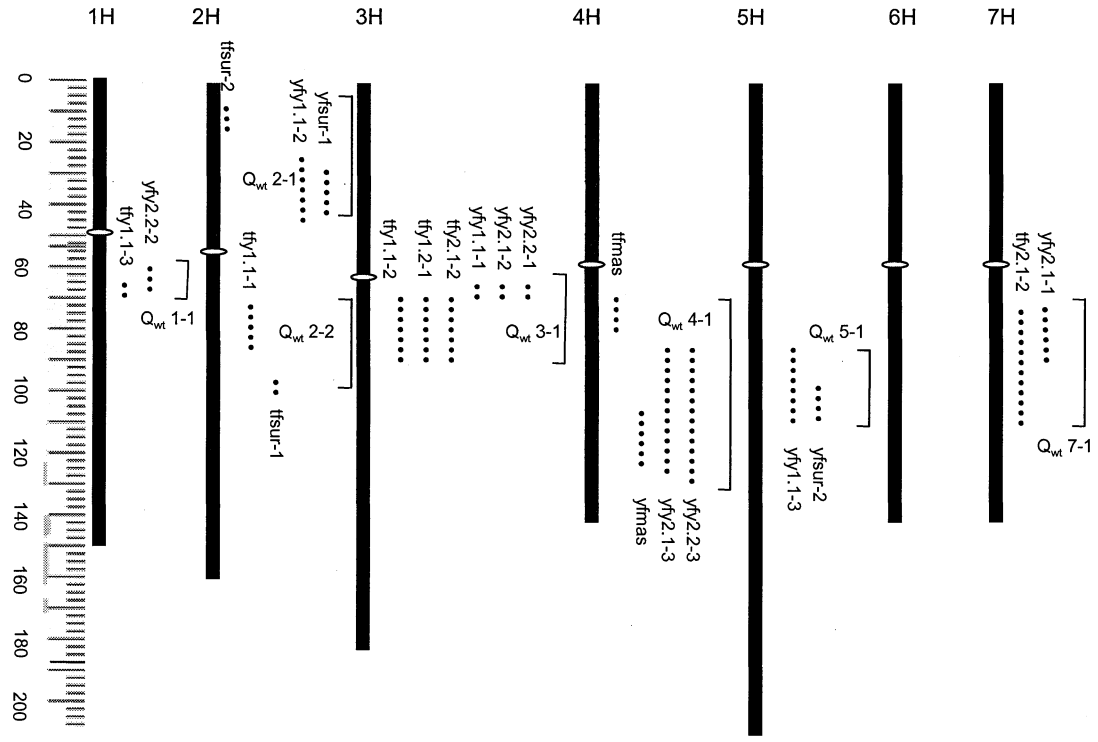


Figure 6.7 Comparison of quantitative trait loci (QTLs) identified for waterlogging tolerance in two different barley doubled haploid populations: tf = Franklin/TX9425; yf = Franklin/Yerong. Flanking markers of each QTL identified in the individual population were re-located on a barley composite map. A general name (such as Qwt1-1) was given to each chromosome region that was associated with different waterlogging tolerance related traits, the first number is the chromosome number, the second number is the serial number of regions identified on that chromosome.

6.4 Discussion

6.4.1 Comparison of QTLs identified for waterlogging tolerance related traits in barley.

Leaf yellowing proportion has been found to be high negatively correlated with grain yield which was regarded as the final criterion for waterlogging tolerance in wheat (van Ginkel 1992). Most of the early studies on waterlogging tolerance in wheat were based on leaf chlorosis or leaf/plant death (Cao et al. 1992, 1994, 1995; Cai et al. 1996). In barley, Hamachi et al (1989) found that screening for waterlogging tolerance by the amount of dead leaves was a useful criterion and the tolerance was under polygenic control, while Setter (1999) concluded that severity of leaf chlorosis was not a good criterion. However, preliminary yield trials using the same genetic material as used in our crosses (unpublished data), showed that under waterlogging conditions, the yield reductions of Franklin (high yellow leaf proportion under waterlogging) and TX9425 (low yellow leaf proportion under waterlogging) were 86% and 28% in a pot experiment and 61% and 39% in a controlled field experiment. Since leaf chlorosis after waterlogging treatment showed high heritability (Chapter 3), this trait was used as the major criterion to test for waterlogging tolerance along with plant survival, plant biomass reduction and seed germination under waterlogging stress in the current study.

The results of one-dimensional QTL analysis (MapQTL 5.0, Van Ooijen et al. 2001) showed that a total of 6 distinct QTLs were identified in the population from Franklin/TX9425, and 6 distinct QTLs were detected in the population of Franklin/Yerong. This conclusion was confirmed by the results of two-dimensional analysis (QTLNetwork 2.0, Yang et al. 2005) by relocating the flanking markers of each QTL on the consensus map constructed in chapter 5 of this thesis. It has also been demonstrated that the QTLs controlling leaf yellowing were very stable under different stress duration and between different experiments, some QTLs controlling leaf chlorosis were co-located with QTLs for other waterlogging related traits such as plant biomass, plant survival, and even with QTLs controlling waterlogging tolerance in barley seed. This result suggested that leaf yellowing proportion (or leaf chlorosis) is an important stable selection criterion for barley waterlogging tolerance (Hamachi et al. 1989) which can be used practically in a barley breeding program.

The QTL analysis of two doubled haploid populations (Figure 6.7) found at least seven distinct QTLs for waterlogging tolerance. It was also demonstrated that some QTLs controlling leaf chlorosis were very stable and were validated under different stress duration, between different experiments and different populations ($Q_{wt}1$-1, $Q_{wt}3$-1, and $Q_{wt}7$-1). Some QTLs affected multiple waterlogging tolerance related traits, for example, QTL $Q_{wt}4$-1 contributed not only to reducing barley leaf chlorosis, but also increasing plant biomass under waterlogging stress, whereas other QTLs such as $Q_{wt}2$-1, $Q_{wt}2$-2 and $Q_{wt}5$-1 controlled both leaf chlorosis and plant survival. This result suggested that leaf chlorosis is an important stable selection criterion for barley waterlogging tolerance which can be used practically in a barley breeding program.

Accuracy of QTL mapping is important in implementing marker-assisted selection (MAS) for polygenic traits, and exact confidence intervals for QTL positions are not easily obtained (Visscher et al. 1996), although typical approximate confidence intervals for QTL positions are of the order of 20 cM (Kearsey et al. 1998; Dekkers et al. 2002). The size of confidence intervals cannot continue to be reduced indefinitely by increasing marker density, so improvement of accuracy of location of QTL positions requires increased mapping population size (Stuber 1998). The results of this study shows that the positions of QTLs identified in the Franklin/Yerong population were more accurate and repeatable than those in the Franklin/TX9425 population by relocating the flanking marker of each QTL in a consensus map. Given the difference in the size of the populations, the effect of QTLs identified in the Franklin/TX9425 population could be overestimated compared to that in the Franklin/Yerong population. However, the high heritability of waterlogging tolerance related traits [14] may alleviate the overestimation of the QTLs identified in Franklin/TX9425.

There is only one published report of QTL for waterlogging tolerance in barley. Qian et al. (2005) found one SSR marker (WMC1E8) correlated with waterlogging tolerance based on content of chlorophyll in the second top leaf developed in a barley F2 population by constructing two DNA (tolerant and susceptible) bulks. The identified marker explained 29.9% of the total variation (Qian et al. 2005), and the authors deduced that this QTL was located on chromosome 1H based on the published barley linkage maps (Scottish Crop Research Institute Barley, 1995). In our study we identified QTLs controlling leaf yellowing proportion in both populations on chromosome 1H. However,

it is difficult to compare the position of the loci between the two studies because there are few shared markers between our map and that of Scottish Crop Research Institute.

6.4.2 Comparison of QTLs controlling waterlogging tolerance in barley with that in other cereal crops.

As has been reviewed in Chapter 2, genetic maps have allowed the identification of chromosomal regions that control some traits related to waterlogging stress response in several cereal species. Different segregating populations of rice, maize, wheat, and barnyard grass have been studied for diverse waterlogging related characteristics or criteria, such as plant survival, leaf senescence, and the extent of stimulation of shoot elongation caused by stress (Toojinda et al. 2003), waterlogged shoot growth and waterlogged root growth (Cakir et al. 2005), adventitious root formation and leaf injury (Mano et al. 2005; Mano et al. 2006). Quantitative trait loci (QTLs) controlling flooding or waterlogging have been identified in these species based on many different traits.

Comparison of genetic mechanisms of waterlogging or flooding tolerance among different crops remains difficult because different waterlogging related traits were used for QTL analysis in these studies. Another difficulty for comparing QTLs identified for waterlogging tolerance in different species is the lack of common markers among different genetic linkage maps, sometimes even among different populations within the same species. Different marker nomenclature among researchers also contributed to the reduced value of these comparative mapping studies.

Comparison of some major QTLs controlling waterlogging or flooding tolerance based on the same sub-traits, however, has provided some interesting information. For example, a major QTL controlling waterlogging tolerance based on dry matter production in maize was located on chromosome 1 (Mano et al. 2006). In our experiment, a QTL controlling plant biomass under waterlogging stress was identified on chromosome 4H. Comparative mapping showed that maize chromosome 1 had a highly homoeologous relationship with chromosome 4 in wheat (Ahn et al. 1993) and 4H in barley (Linde-Laursen et al. 1997).

The QTLs controlling rice percent plant survival under submergence stress were mapped to chromosome 7, 9, and 10, and the QTL located on chromosome 9 was the most significant one. In barley, the QTLs contributing to plant survival were located on

chromosomes 2H and 5H. According to comparative mapping in the grass family, rice chromosome 2 had a homologous relationship with wheat chromosome 5L and chromosome 2 in maize (Ahn et al. 1993). Comparison of homologous relations between wheat and maize showed that maize chromosome 2 also corresponds to chromosome 2 in wheat (Ahn et al. 1993), so it can be deduced that rice chromosome 9 is homologous with barley chromosome 2H and 5H (Linde-Laursen et al. 1997).

The major QTLs controlling leaf senescence have been identified in rice and maize, they were located on chromosome 9 and chromosome 1 in the rice and maize genome, respectively. The QTLs controlling barley flooding tolerance based on leaf chlorosis have also been identified in this study, two major QTLs were located on chromosome 3H and 4H. The maize chromosome 1 corresponded to chromosome 4 and 3L in wheat (Ahn et al. 1993), hence corresponding to chromosome 4H and 3H. However, no homologous relationship between rice chromosome 9 and barley chromosome 3H and 4H can be deduced from the comparative mapping.

These comparisons demonstrated that QTLs controlling leaf senescence and dry matter production in maize and barley could be homoalleles in different genetic backgrounds. Similarly, the QTLs controlling plant survival in barley could also be the corresponding allele controlling plant survival in rice. However, the QTLs controlling leaf senescence in barley are different from that in rice because that were mapped to chromosomes with no known homologous relationships.

6.4.3 Comparison of genetic mechanisms of barley waterlogging tolerance with some other abiotic stress tolerances in barley.

Comparison of genetic mechanisms of barley responding to different soil-based abiotic stresses, such as drought, salinity, and metal toxicity with that to waterlogging stress may provide us with fundamental knowledge on how to improve waterlogging tolerance in barley. These abiotic stresses are often occurring simultaneously or sequentially (Ashraf et al. 2004).

QTL analysis of physiological traits and agronomic traits related to drought tolerance have been reported from controlled environment and field data (Teulat et al. 1997a, 1998, 2001a,b, 2002; Teulat-Merah et al. 2000, 2003; This et al. 2000). The results of these

studies showed that four hot spots for physiological traits overlapping with agronomic traits were on chromosome 2H (centromeric region), 4H (long arm), 6H (long arm) and 7H (centromeric region). The hot spot on 4H showed an association with grain yield, but the others were associated with important yield components such as number of grain per spike, number of fertile tillers, dry aerial biomass and harvest index. Most associations of physiological QTLs were with agronomic QTLs for thousand grain weight. Other interesting physiological/agronomic associations were found on chromosome 5H between carbon isotope discrimination and thousand grain weight, and on chromosome 6H between carbon isotope discrimination and several yield components, including thousand grain weight. Foster et al (2004) identified significant QTLs for each drought tolerance related trait. For plant height, twelve different chromosome regions were identified. The largest effect, which was common across environments, was on chromosome 3H in a region containing the semi-dwarf gene, *sdw1*. Four QTLs for relative water content were located on chromosome 2H and 7H. Two QTLs influencing heading date were detected on chromosome 3H and 7H. A large number of QTLs were found for grain yield, the positive alleles for increasing yield were mainly contributed by *H. vulgare*, but in five cases, the positive alleles originated from *H. spontaneum*. Two of these *H. spontaneum* QTLs were detected across the environments and located on chromosome 2H and 5H (Foster et al. 2004).

Sixteen primary QTLs controlling salt tolerance were revealed in a barley doubled haploid population (Ellis et al. 2002). Twelve QTLs were identified for seedling traits and four were for mature plant traits and plot yield. The largest individual QTL effects were associated with the chromosomal regions around the two dwarfing genes sdw1 (3H) and ari-e.GP (5H) that were segregating in the DHs. Among the 16 QTLs identified, 7 QTLs were co-located with the dwarfing genes *sdw1*, on chromosome 3H, and *ari-e*.GP, on chromosome 5H, including seedling leaf response to gibberellic acid (GA3). QTLs controlling the growth of leaves on chromosome 2H and 3H and emergence of tillers and grain yield were independent of the dwarfing genes (Ellis et al. 2002). Dadshani et al. (2004) recently evaluated the Oregon Wolfe Barley (OWB) mapping population for identification of QTLs for salt tolerance traits. Germination and early seedling growth were used to evaluate the salinity tolerance expression in the mapping population. Three chromosome regions were found to correlate with salinity evaluation scores of the OWB

population. Two were located on chromosome 5H and one on chromosome 7H (Dadshani et al. 2004). The QTLs for salt tolerance at germination have also been described for the Harrington / TR306 population on chromosomes 1H and 5H and for the Steptoe / Morex population on chromosomes 4H, 5H and 6H (Mano et al. 1997).

QTL studies have shown that the Al tolerance gene (*Alp2*) in Yambla/WB229 population is located on the long arm of chromosome 4H (Raman et al. 2001) and a similar genomic region is involved for tolerance (*Alp*) in Dayton/Harlan Hybrid population (Minella and Sorrells, 1997; Tang et al. 2000). The results of an F_2 population of Harrington / Brindabella indicated that a major QTL, controlled by the Al tolerant parent Brindabella, was identified on *4H*. Microsatellite markers Bmac186 and Bmac310 showed a highly significant association with the Al tolerance gene and therefore can be used as markers for selection of the Al tolerance gene from Brindabella (*Alp3*) (Raman et al. 2003). It appears that homologous genomic regions may be involved in aluminium tolerance in barley.

Comparison of the QTLs previously identified in barley for the tolerance to drought, salinity, and aluminium with the QTLs identified for tolerance to waterlogging stress in this study gives some interesting information. Among the seven barley chromosomes, chromosomes 3H, 4H and 5H carry QTLs for tolerance to all of the four abiotic stresses mentioned above, and chromosome 2H only carries QTLs for waterlogging and drought tolerance. Chromosomes 1H, 6H, and 7H carry QTLs for three of the above stresses, no QTLs for waterlogging tolerance were mapped on barley chromosome 6H, and no QTLs for aluminium tolerance were mapped on chromosome 1H and 7H. However, in respect of QTL effects, chromosomes 2H, 3H, 4H, 5H and 7H often carry QTLs with large effects of tolerance, chromosomes 1H and 6H seem less important for abiotic stress tolerance. The comparison also showed that QTLs on chromosome 3H for drought tolerance (Forster et al. 2004) and that for salt tolerance (Ellis et al. 2002) were collated with the dwarfing gene *sdw1*. Compare to other chromosomes, 3H also carries the largest number of QTLs for waterlogging tolerance in barley, and most QTLs on this chromosome were co-located in the same region. It seems that chromosome 3H contains genes or QTLs with multi-effects for different abiotic stresses.

6.4.4 Identification of molecular markers with high potential in barley breeding programs by integration of genetic information gained from individual mapping studies.

As it has been demonstrated in this study, and in other previously published studies (Jaccoud et al. 2001), diversity array technology (DArT) is very efficient for whole-genome profiling (Wenzl et al. 2004). Due to its non-dependence on sequencing information, high throughput, low cost, and the ability to easily convert them into other types of markers, DArT could enhance the utility of marker assisted selection in plant breeding programs (Wenzl et al 2006). However, this technique is still limited to only a few labs at this stage, and most plant breeders can not access this marker system. In order to provide plant breeders with practically useful molecular markers in improving barley waterlogging tolerance, a barley consensus map was constructed to link DArT markers with some SSR and RFLP markers which have been previously developed and applied widely in barley mapping studies.

QTLs controlling waterlogging tolerance in barley have been identified in this study, and the flanking markers of each QTL were relocated on a consensus map constructed using four different barley DH populations (Chapter 5). Some SSR or RFLP markers closely linked to these QTLs were selected. If barley breeders can access the SSR and RFLP markers that are associated with the genes or QTLs identified in this study, then these genes or QTLs would allow for marker assisted selection (MAS). In the future it is expected that DArT will be more widely applied, given its ease of use compare to other types of markers (Jaccoud et al 2001; Wenzl et al. 2004, 2006).

Improving waterlogging tolerance in barley is at an early stage compared with other traits, but could benefit more from MAS efficiencies. The potential to use MAS in combination with traditional field selection will significantly enhance the progress in improving barley waterlogging tolerance in barley.

Chapter 7 General discussions and conclusions

7.1 Quantitative inheritance of waterlogging tolerance in barley

A 6 X 6 half diallel analysis was conducted in barley from crosses of three waterlogging tolerant Chinese cultivars and three susceptible Australian or Japanese cultivars to investigate the genetic control of waterlogging. Leaf chlorosis (leaf yellowing proportion) was used as the indicator of waterlogging tolerance. High heritability (h^2_B = h^2_N = 0.73) of waterlogging tolerance indicated that selection in early generations could be very efficient. However, results from chapter 6 suggested that waterlogging tolerance was complicated since the heritability for this trait varied between populations and experiments. The continuous distribution of waterlogging tolerance in a doubled haploid (DH) population generated from a cross between TX9425 (tolerant) and Naso Nijo (susceptible) in chapter 3 and other two populations in chapter 6 indicated that the tolerance was likely to be controlled by multiple genes, which is consistent with the earlier report by Hamachi et al (1989) but different from the results in wheat (Cao et al. 1992, 1995), in which a single gene was involved in waterlogging tolerance based on leaf chlorosis. The complicated characteristics of waterlogging tolerance in barley have made QTL identification and marker-assisted selection more valuable in barley breeding programs.

7.2 Validation of the robustness of DArT in a large mapping population

DArT is a new high throughput microarray-based marker system. The microarray platform makes genotyping very efficient because all markers on a particular DArT array are scored simultaneously (Jaccoud et al. 2001; Wenzl et al. 2004). However, due to the fact that all the reported experiments using DArT have been based on studies with a sample size of less than 96 (one microtiter plate), validation of the robustness of the marker system was undertaken by DArT genotyping individuals of the same DH population in two separate batches, constructing separate linkage maps and comparing the co-linearity of the markers in each map. The result showed that although one more linkage group was obtained in the smaller batch (88 compared to 92 individuals), all of the 496 common markers assayed over the two sets of progenies were assigned to exactly

the same barley chromosomes. The marker order was highly similar between the two maps with only minor rearrangements of marker orders at intervals of less than 5 cM. These comparisons demonstrated that DArT was very robust when it was used for genotyping a large number of individuals across different assays. The feature of high-throughput and independence from sequence information (Wenzl 2006), together with robustness should enable routine use of DArT markers in plant breeding programs.

7.3 Construction of linkage maps in two barley mapping population

For the purpose of identification of quantitative trait loci controlling waterlogging tolerance in barley, two linkage maps were constructed using two different DH populations. The linkage map constructed using 92 DH lines from the cross between TX9425/Franklin comprised of 412 DArT, 27 SSR and 81 AFLP markers which were organized into 8 linkage groups, instead of the expected 7 groups, because chromosome 6H was split into two groups. The linkage map constructed using 180 DH lines from Yerong/Franklin was based on 496 DArT and 22 SSR markers, which were assigned to 9 linkage groups with chromosomes 1H and 2 H split into two fragments. The total length of these linkage maps was similar to some AFLP linkage maps with total map lengths of 900-1100 cM (Powell et al. 1997; Yin et al. 1999), but was shorter than other barley linkage maps mainly based on RFLP or SSR markers with total lengths of 1100-1300 cM (Kleinhofs et al. 1993; Ramsay et al 2000). It has been reported that maps constructed using Joinmap are often shorter than those constructed using MAPMAKER (Sewell et al.1999 in loblolly pine and Qi et al.1996 in barley). The size difference between this map and other published maps may also result from lower genome homology between the two parents of this cross, reducing recombination frequency in the F1 and map size (Bonierbale et al. 1988; Gebhardt et al. 1991; Vaillancourt and Slinkard 1993; Paillard et al. 1996).

7.4 Construction of a new barley consensus map

For the purpose of integrating the genetic information contained in different linkage maps constructed using DArT, SSR and RFLP markers, a consensus map was constructed using four barley crosses. The consensus map comprised 2,111 markers arranged into seven linkage groups. It spanned a total length of 1,136 cM. The marker density of this map was considerably higher than that in previously published barley consensus maps

(Langridge et al. 1995; Qi et al. 1996; Karakousis et al. 2003; Diab et al. 2006). Such a consensus map could serve as a useful tool for molecular breeding in barley and as a basis for studies of genome organization and evolution. For example, many more markers showed segregation distortion than expected. Out of the 2975 markers used across all four populations 21.1%, 10.9%, and 7.9% exhibited segregation distortion at 5%, 1%, and 0.5% probability threshold respectively. DArT markers were not more likely to show segregation distortion than other marker types. Of the 635 markers showing aberrant segregation in the four populations, 459 markers were located in 16 putative segregation distortion regions (SDR). The SDRs were identified on all seven barley chromosomes, but they were unevenly distributed over the seven chromosomes and their size varied from 4 to 46 cM. Ten of the SDRs were found in at least two populations and several at a consistent map location over the four populations. Further studies are needed to determine the molecular basis of segregation distortion.

7.5 Characterization of quantitative trait loci (QTLs) associated with waterlogging tolerance in barley

In chapter 6 of this thesis, the identification and validation of QTLs controlling waterlogging tolerance in barley was reported. QTL analysis showed that a total of 6 distinct QTLs were identified for the waterlogging related traits in the Franklin/TX9425 population, and 6 QTLs were detected in the population of Franklin/Yerong. In addition, another QTL was detected which operated through the interactions between two different loci. By relocating the flanking markers of each QTL onto the consensus map, comparative mapping of QTLs between two doubled haploid populations (Figure 6.7) found at least seven distinct QTLs for waterlogging tolerance. It was also demonstrated that some QTLs controlling leaf chlorosis were very stable and were validated under different stress duration, between different experiments and different populations (Q_{wt}1-1, Q_{wt}3-1, and Q_{wt}7-1). Some QTLs affected multiple waterlogging tolerance related traits, for example, QTL Q_{wt}4-1 contributed not only to reducing barley leaf chlorosis, but also increasing plant biomass under waterlogging stress, whereas other QTLs such as Q_{wt}2-1, Q_{wt}2-2 and Q_{wt}5-1 controlled both leaf chlorosis and plant survival. This result suggested that leaf chlorosis is an important stable selection criterion for barley waterlogging tolerance which can be used practically in a barley breeding program. Further experiments are needed to identify other and validate the waterlogging

tolerance QTLs we discovered and explore the stability and yield benefits of these QTLs in field experiments. However, it is clear that marker-assisted selection could be a very valuable tool for improving waterlogging tolerance in barley.

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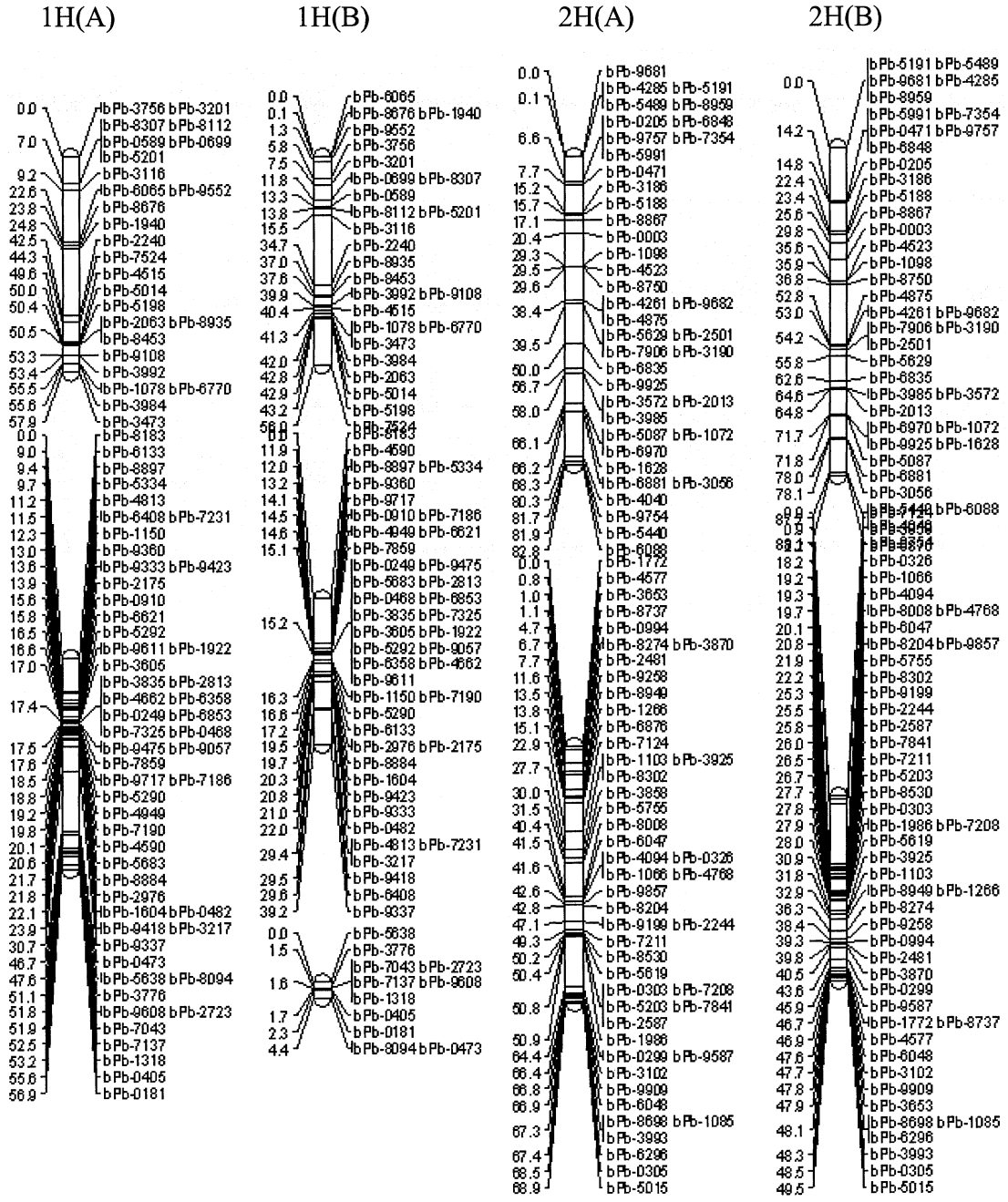
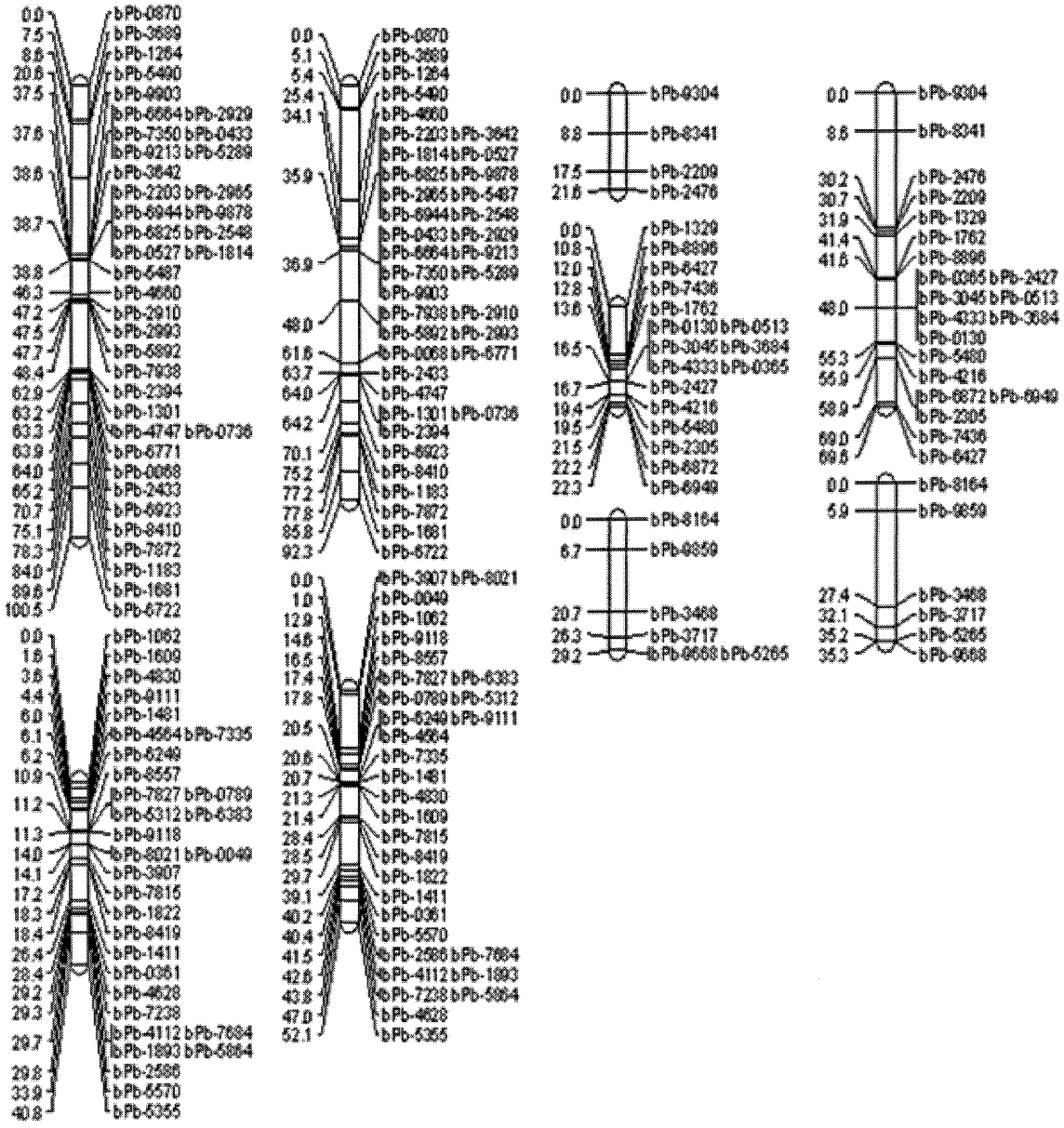
Appendices

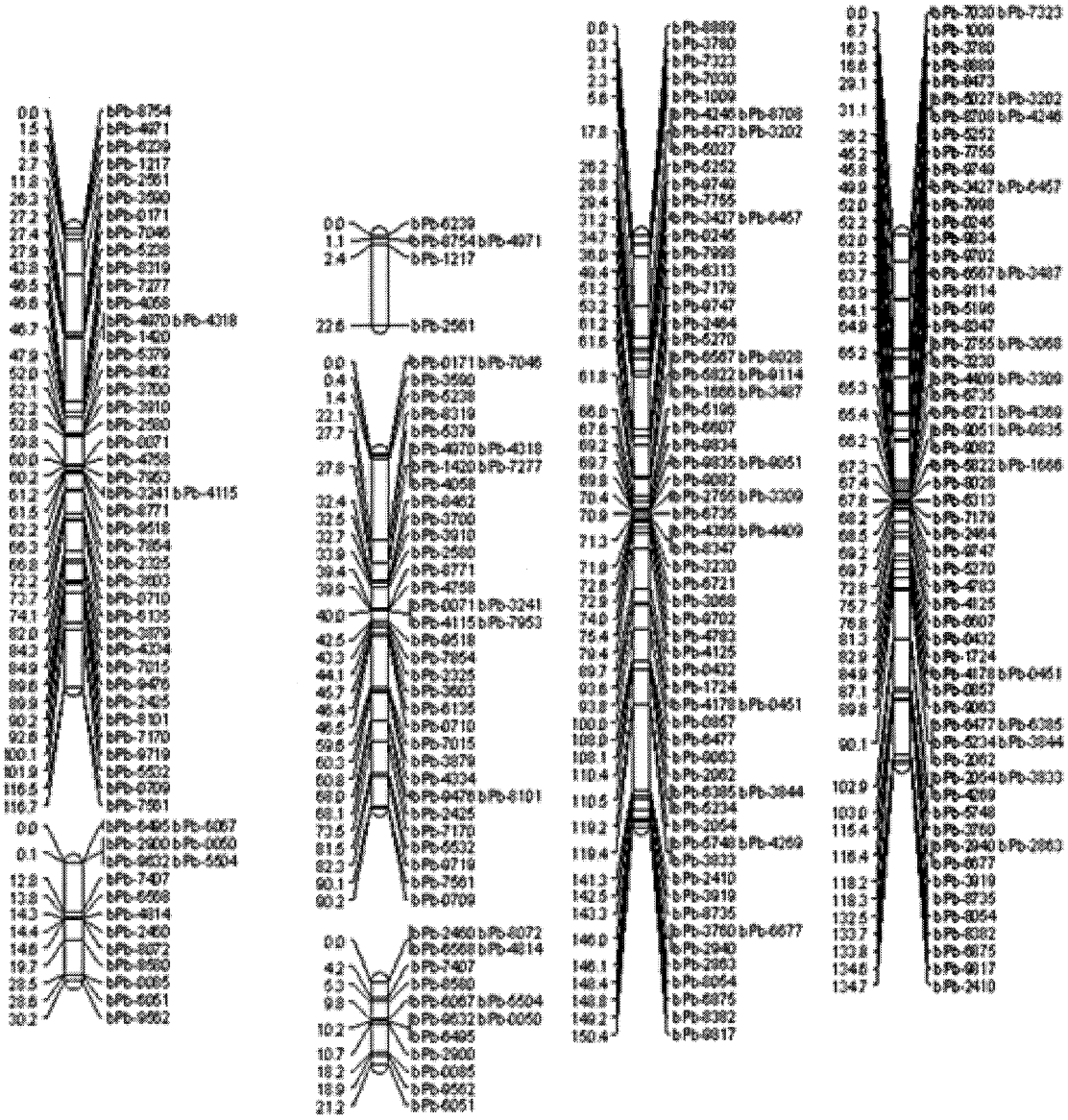
Appendix 1. Colinearity of the genetic linkage maps based on two different sets of progenies (experiment A and B) from Franklin / Yerong population using only DArT markers. The linkage groups associated with a barley chromosome were placed one on top of another and placed side by side for the two experiments based on the linkage knowledge from the overall analysis for this population. Distance between markers is indicated on left side of each linkage group in cM.

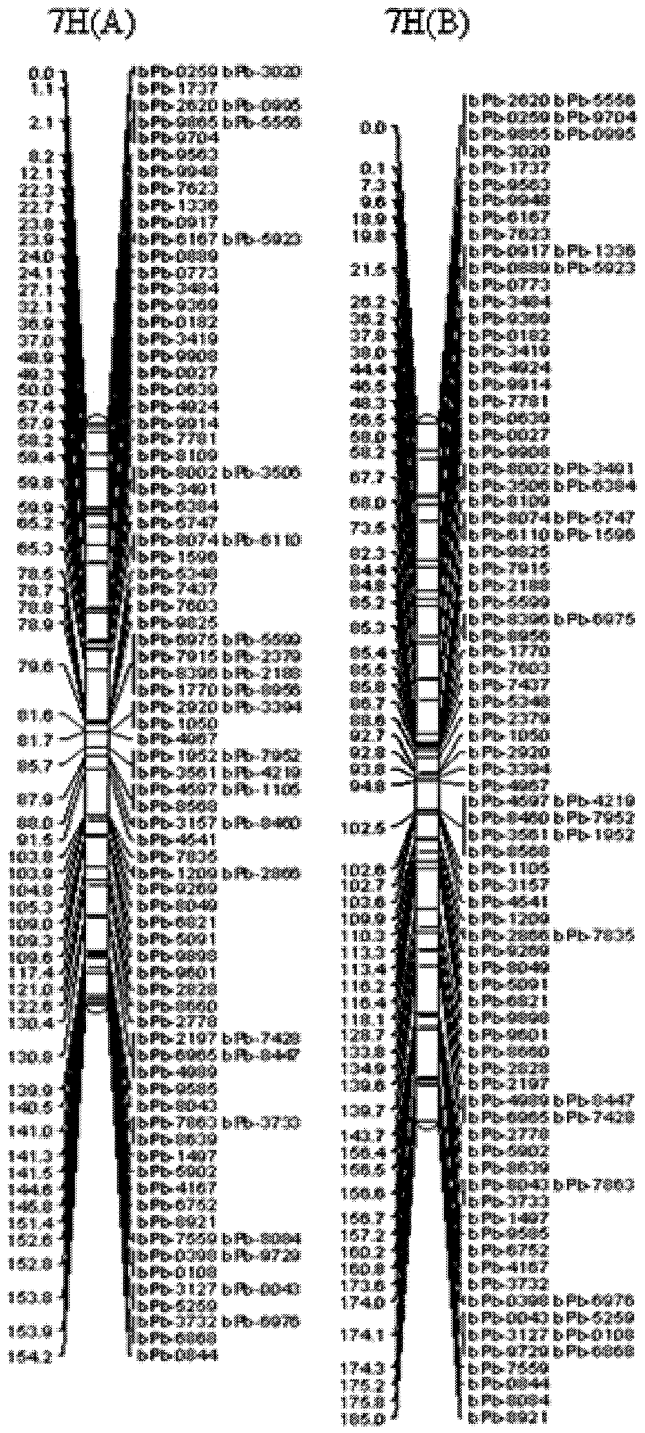


3H(A) 3H(B) 4H(A) 4H(B)

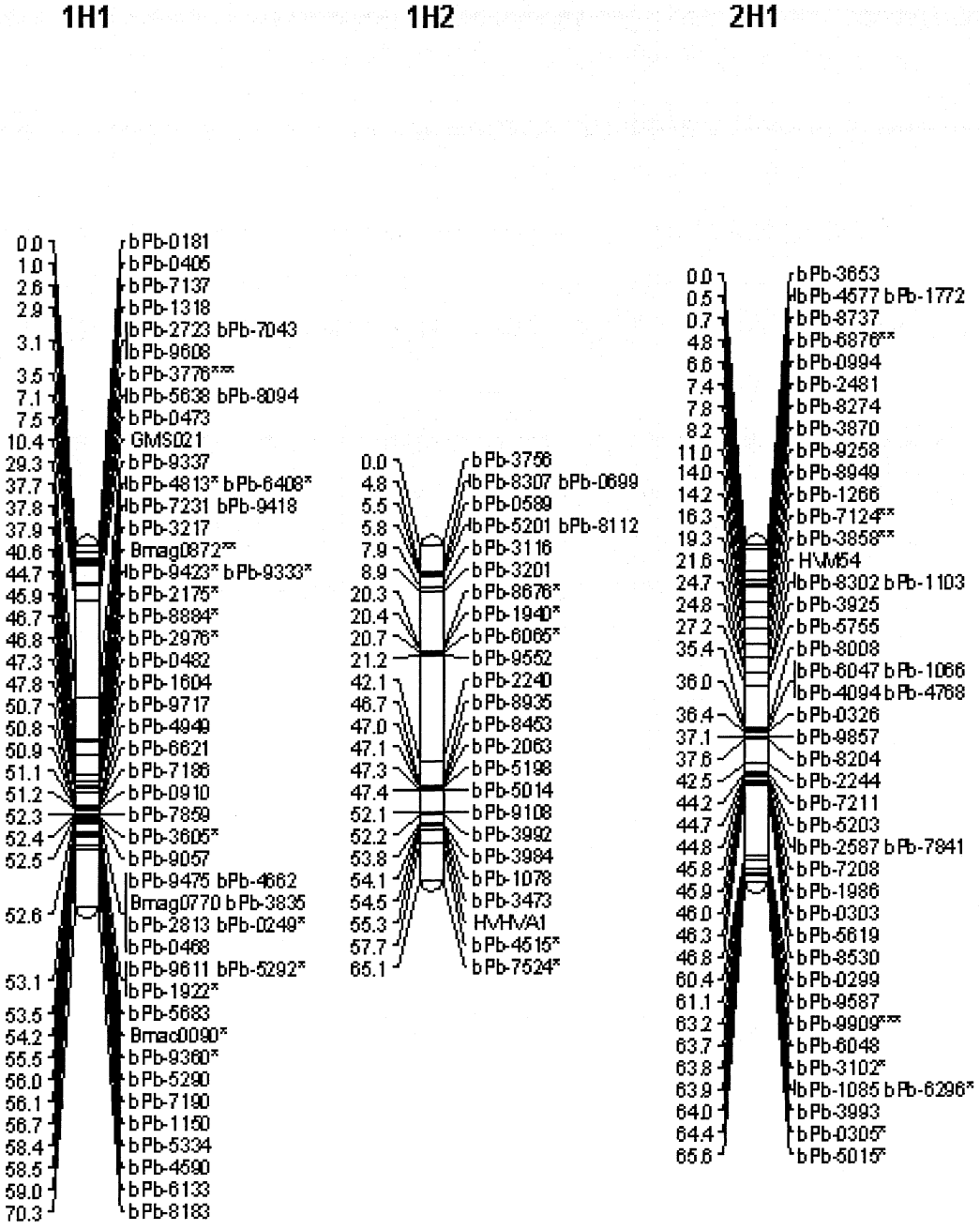


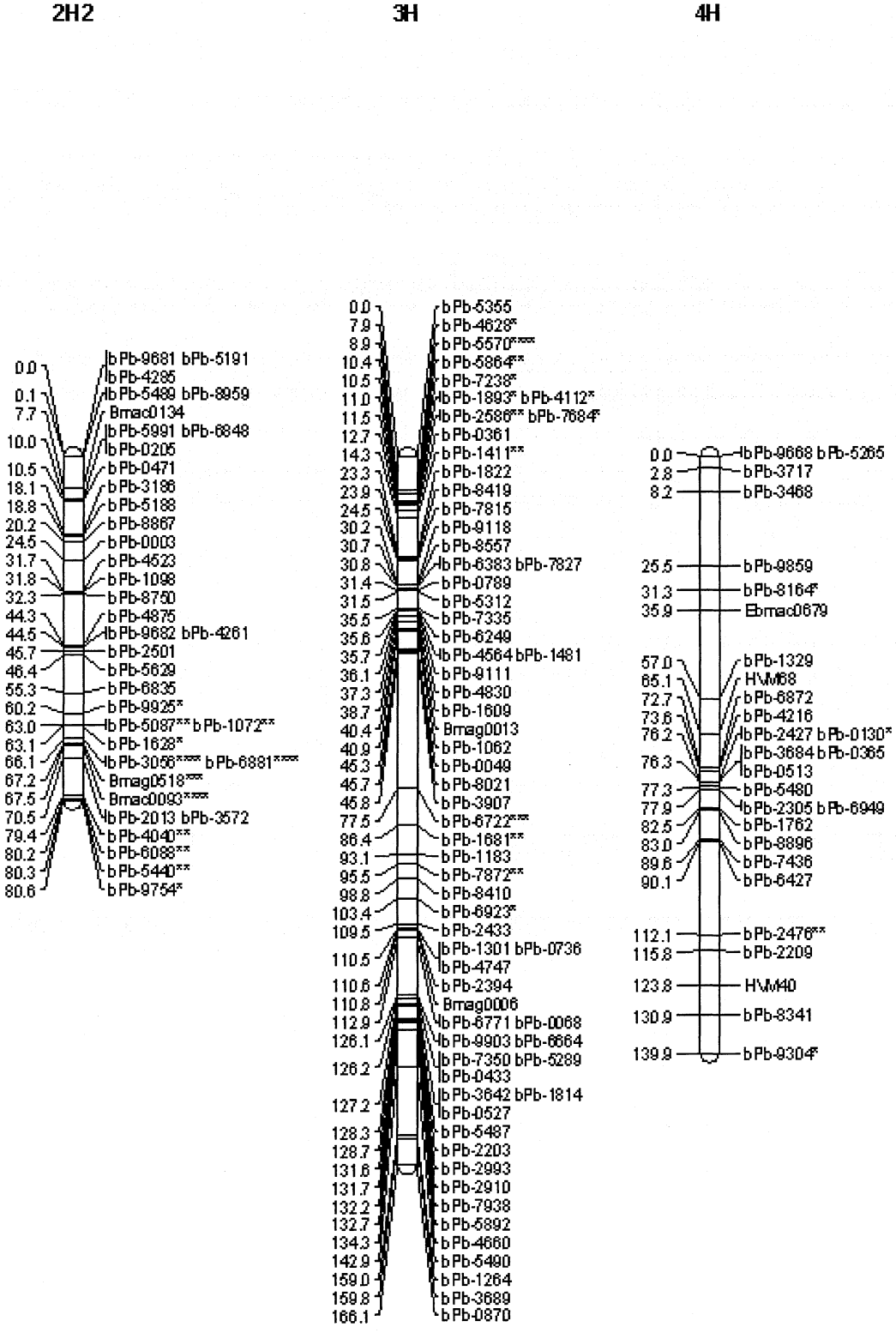
5H(A) 5H(B) 6H(A) 6H(B)

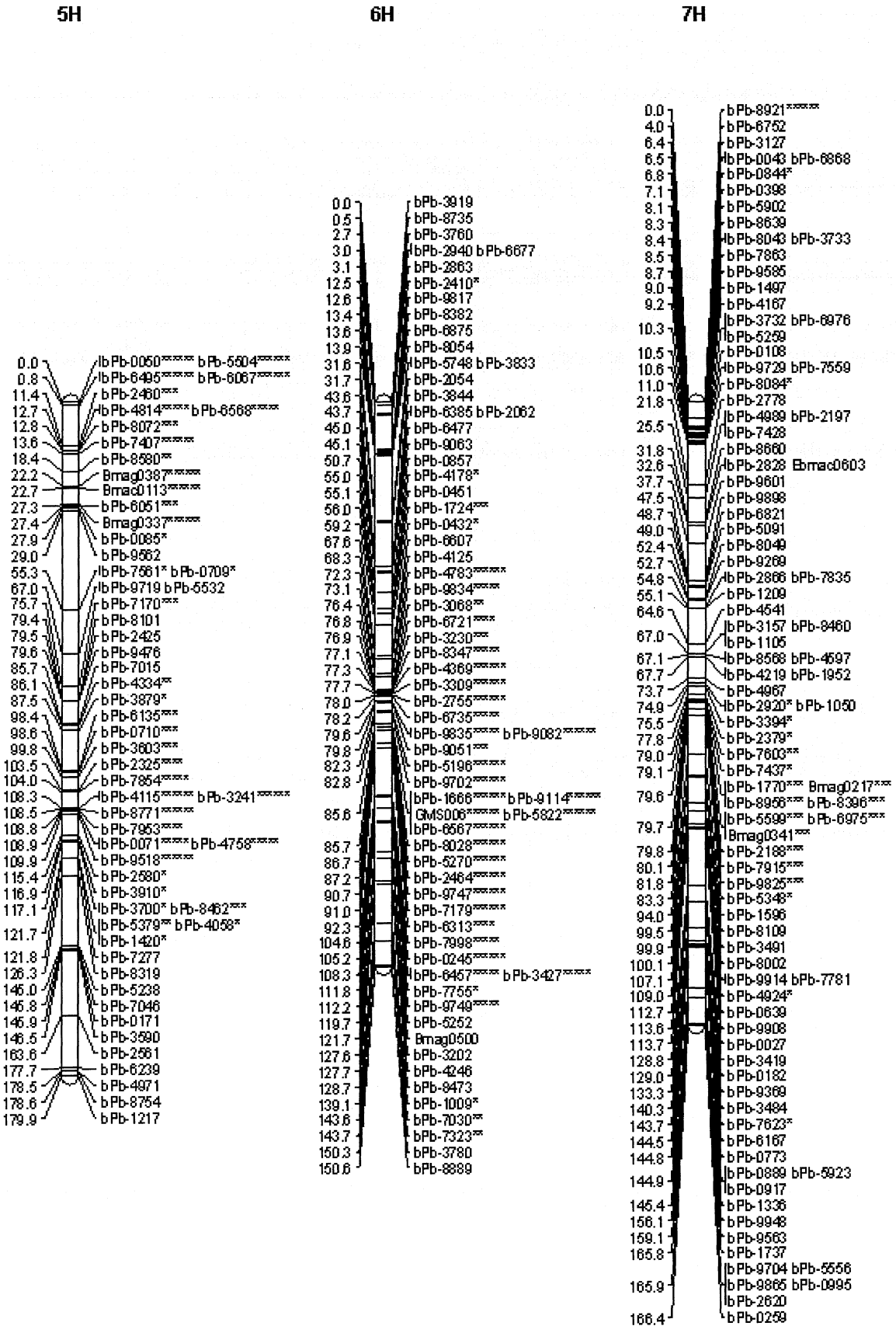



7H(A)
7H(B)

Appendix 2. The overall linkage map based on 180 DH progenies from Franklin / Yerong population using DArT and microsatellite markers. Markers with segregation distortion were indicated with stars (*), with P < 0.05 = *; P < 0.01 = **; P < 0.005 = ***. The linkage groups are named and organized by chromosomes, e.g. chromosome 1H had two linkage groups 1H1 and 1H2. DArT marker are encoded with the letter bPb followed by a number showing the position of marker (clone) in the library, the other markers are all microsatellites.




2H2
0.0 bPb-9681 bPb-5191
bPb-4285
0.1 bPb-5489 bPb-8959
7.7 Bmac0134
10.0 bPb-5991 bPb-6848
bPb-0205
10.5 bPb-0471
18.1 bPb-3186
18.8 bPb-5188
20.2 bPb-8867
24.5 bPb-0003
31.7 bPb-4523
31.8 bPb-1098
32.3 bPb-8750
44.3 bPb-4875
44.5 bPb-9682 bPb-4261
45.7 bPb-2501
46.4 bPb-5629
55.3 bPb-6835
60.2 bPb-9925*
63.0 bPb-5087** bPb-1072**
63.1 bPb-1628*
66.1 bPb-3056**** bPb-6881****
67.2 Bmag0518***
67.5 Bmac0093****
70.5 bPb-2013 bPb-3572
79.4 bPb-4040**
80.2 bPb-6088**
80.3 bPb-5440**
80.6 bPb-9754*
3H
0.0 bPb-5355
7.9 bPb-4628*
8.9 bPb-5570****
10.4 bPb-5864**
10.5 bPb-7238*
11.0 bPb-1893* bPb-4112*
11.5 bPb-2586** bPb-7684*
12.7 bPb-0361
14.3 bPb-1411**
23.3 bPb-1822
23.9 bPb-8419
24.5 bPb-7815
30.2 bPb-9118
30.7 bPb-8557
30.8 bPb-6383 bPb-7827
31.4 bPb-0789
31.5 bPb-5312
35.5 bPb-7335
35.6 bPb-6249
35.7 bPb-4564 bPb-1481
36.1 bPb-9111
37.3 bPb-4830
38.7 bPb-1609
40.4 Bmag0013
40.9 bPb-1062
45.3 bPb-0049
45.7 bPb-8021
45.8 bPb-3907
77.5 bPb-6722***
86.4 bPb-1681**
93.1 bPb-1183
95.5 bPb-7872**
98.8 bPb-8410
103.4 bPb-6923*
109.5 bPb-2433
110.5 bPb-1301 bPb-0736
bPb-4747
110.6 bPb-2394
110.8 Bmag0006
112.9 bPb-6771 bPb-0068
126.1 bPb-9903 bPb-6664
126.2 bPb-7350 bPb-5289
bPb-0433
127.2 bPb-3642 bPb-1814
bPb-0527
128.3 bPb-5487
128.7 bPb-2203
131.6 bPb-2993
131.7 bPb-2910
132.2 bPb-7938
132.7 bPb-5892
134.3 bPb-4660
142.9 bPb-5490
159.0 bPb-1264
159.8 bPb-3689
166.1 bPb-0870
4H
0.0 bPb-9668 bPb-5265
2.8 bPb-3717
8.2 bPb-3468
25.5 bPb-9859
31.3 bPb-8164*
35.9 Ebmac0679
57.0 bPb-1329
65.1 HVM68
72.7 bPb-6872
73.6 bPb-4216
76.2 bPb-2427 bPb-0130*
76.3 bPb-3684 bPb-0365
bPb-0513
77.3 bPb-5480
77.9 bPb-2305 bPb-6949
82.5 bPb-1762
83.0 bPb-8896
89.6 bPb-7436
90.1 bPb-6427
112.1 bPb-2476**
115.8 bPb-2209
123.8 HVM40
130.9 bPb-8341
139.9 bPb-9304*


5H
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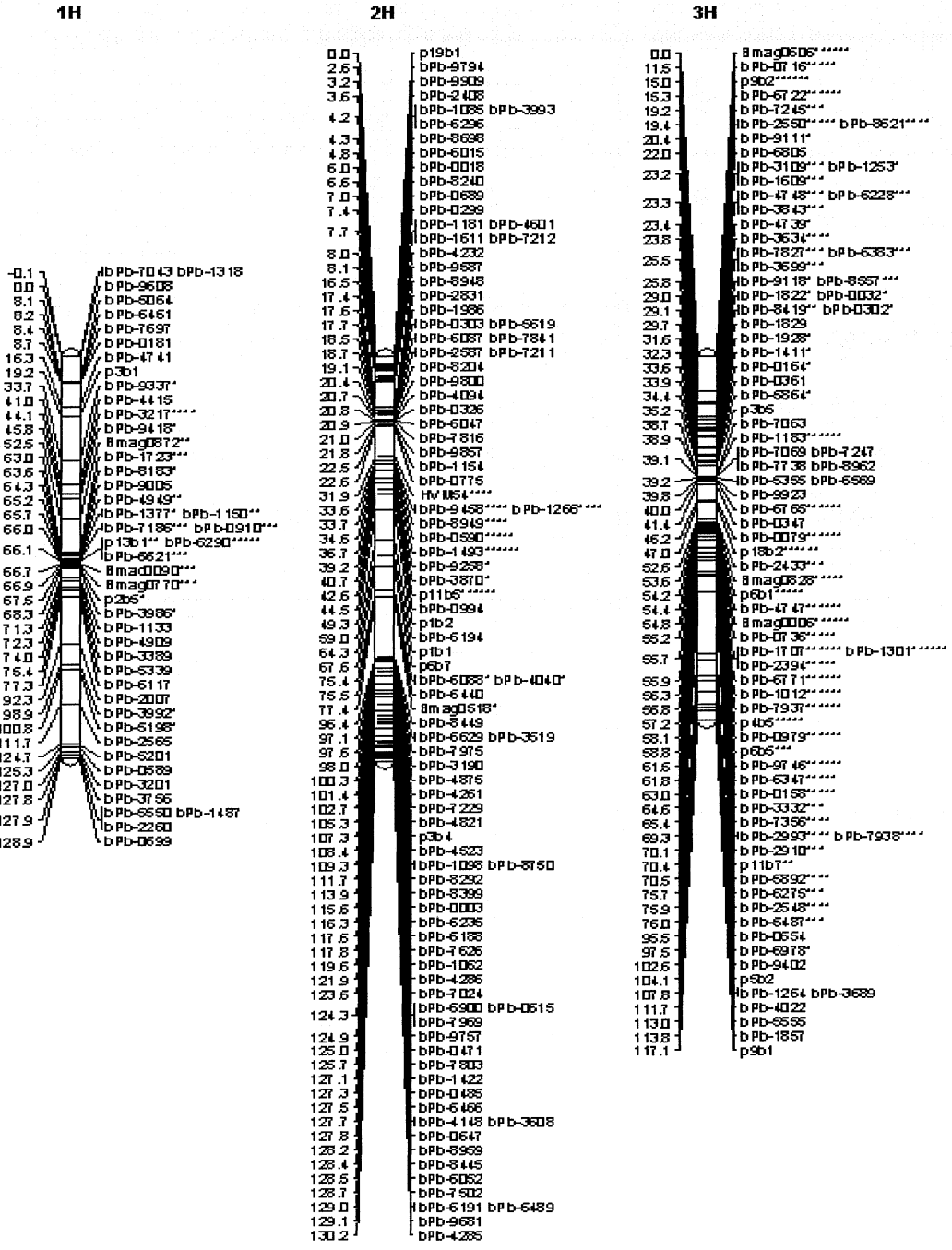
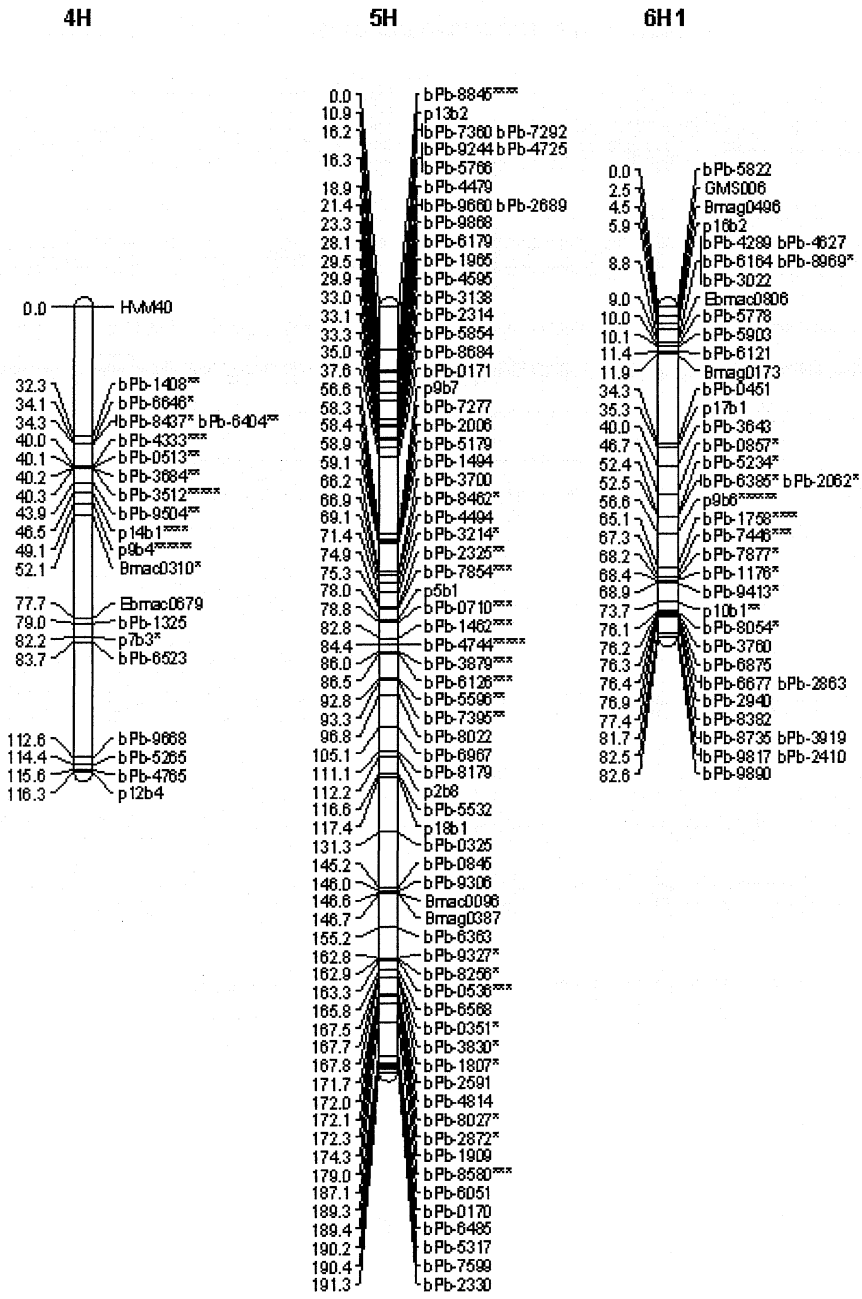
12.7 bPb-4814***** bPb-6568*****
12.8 bPb-8072***
13.6 bPb-7407*****
18.4 bPb-8580***
22.2 Bmag0387*****
22.7 Bmac0113*****
27.3 bPb-6051***
27.4 Bmag0337*****
27.9 bPb-0085*
29.0 bPb-9562
55.3 bPb-7561* bPb-0709*
67.0 bPb-9719 bPb-5532
75.7 bPb-7170***
79.4 bPb-8101
79.5 bPb-2425
79.6 bPb-9476
85.7 bPb-7015
86.1 bPb-4334**
87.5 bPb-3879*
98.4 bPb-6135***
98.6 bPb-0710***
99.8 bPb-3603***
103.5 bPb-2325*****
104.0 bPb-7854*****
108.3 bPb-4115***** bPb-3241*****
108.5 bPb-8771*****
108.8 bPb-7953*****
108.9 bPb-0071***** bPb-4758*****
109.9 bPb-9518*****
115.4 bPb-2680*
116.9 bPb-3910*
117.1 bPb-3700** bPb-8462***
121.7 bPb-5379** bPb-4058*
bPb-1420*
121.8 bPb-7277
126.3 bPb-8319
145.0 bPb-5238
145.8 bPb-7046
145.9 bPb-0171
146.5 bPb-3590
163.6 bPb-2561
177.7 bPb-6239
178.5 bPb-4971
178.6 bPb-8754
179.9 bPb-1217
6H
0.0 bPb-3919
0.5 bPb-8735
2.7 bPb-3760
3.0 bPb-2940 bPb-6677
3.1 bPb-2863
12.5 bPb-2410*
12.6 bPb-9817
13.4 bPb-8382
13.6 bPb-6875
13.9 bPb-8054
31.6 bPb-5748 bPb-3833
31.7 bPb-2054
43.6 bPb-3844
43.7 bPb-6385 bPb-2062
45.0 bPb-6477
45.1 bPb-9063
50.7 bPb-0857
55.0 bPb-4178*
55.1 bPb-0451
56.0 bPb-1724***
59.2 bPb-0432*
67.6 bPb-6607
68.3 bPb-4125
72.3 bPb-4783*****
73.1 bPb-9834*****
76.4 bPb-3068**
76.8 bPb-6721****
76.9 bPb-3230***
77.1 bPb-8347*****
77.3 bPb-4369*****
77.7 bPb-3309*****
78.0 bPb-2755*****
78.2 bPb-6735*****
79.6 bPb-9835***** bPb-9082*****
79.8 bPb-9051***
82.3 bPb-5196*****
82.8 bPb-9702*****
bPb-1666***** bPb-9114*****
85.6 GMS006***** bPb-5822*****
bPb-6567*****
85.7 bPb-8028*****
86.7 bPb-5270*****
87.2 bPb-2464*****
90.7 bPb-9747*****
91.0 bPb-7179*****
92.3 bPb-6313*****
104.6 bPb-7998*****
105.2 bPb-0245*****
108.3 bPb-6457***** bPb-3427*****
111.8 bPb-7755*
112.2 bPb-9749*****
119.7 bPb-5252
121.7 Bmag0500
127.6 bPb-3202
127.7 bPb-4246
128.7 bPb-8473
139.1 bPb-1009*
143.6 bPb-7030**
143.7 bPb-7323**
150.3 bPb-3780
150.6 bPb-8889
7H
0.0 bPb-8921*****
4.0 bPb-6752
6.4 bPb-3127
6.5 bPb-0043 bPb-6868
6.8 bPb-0844*
7.1 bPb-0398
8.1 bPb-5902
8.3 bPb-8639
8.4 bPb-8043 bPb-3733
8.5 bPb-7863
8.7 bPb-9585
9.0 bPb-1497
9.2 bPb-4167
10.3 bPb-3732 bPb-6976
bPb-5259
10.5 bPb-0108
10.6 bPb-9729 bPb-7559
11.0 bPb-8084*
21.8 bPb-2778
25.5 bPb-4989 bPb-2197
bPb-7428
31.8 bPb-8660
32.6 bPb-2828 Ebmac0603
37.7 bPb-9601
47.5 bPb-9898
48.7 bPb-6821
49.0 bPb-5091
52.4 bPb-8049
52.7 bPb-9269
54.8 bPb-2866 bPb-7835
55.1 bPb-1209
64.6 bPb-4541
67.0 bPb-3157 bPb-8460
bPb-1105
67.1 bPb-8568 bPb-4697
67.7 bPb-4219 bPb-1952
73.7 bPb-4967
74.9 bPb-2920* bPb-1050
75.5 bPb-3394*
77.8 bPb-2379*
79.0 bPb-7603**
79.1 bPb-7437*
79.6 bPb-1770*** Bmag0217***
bPb-8956*** bPb-8396***
bPb-5599*** bPb-6975***
79.7 Bmag0341***
79.8 bPb-2188***
80.1 bPb-7915***
81.8 bPb-9825***
83.3 bPb-5348*
94.0 bPb-1596
99.5 bPb-8109
99.9 bPb-3491
100.1 bPb-8002
107.1 bPb-9914 bPb-7781
109.0 bPb-4924*
112.7 bPb-0639
113.6 bPb-9908
113.7 bPb-0027
128.8 bPb-3419
129.0 bPb-0182
133.3 bPb-9369
140.3 bPb-3484
143.7 bPb-7623*
144.5 bPb-6167
144.8 bPb-0773
144.9 bPb-0889 bPb-5923
bPb-0917
145.4 bPb-1336
156.1 bPb-9948
159.1 bPb-9563
165.8 bPb-1737
165.9 bPb-9704 bPb-5556
bPb-9865 bPb-0995
bPb-2620
166.4 bPb-0259

Appendix 3. Barley genetic linkage map of Franklin / TX9425 population based on DArT, AFLP and SSR markers. Markers with segregation distortion were indicated with stars (*), with P < 0.05 = *; P < 0.01 = **; P < 0.005 = ***. The linkage groups are named and organized by chromosomes, e.g. chromosome 1H had two linkage groups 1H1 and 1H2. DArT markers are encoded with the letter bPb followed by a number; AFLP markers were named using a code for each primer combination followed by sequential numbers for scored bands; other markers are microsatellites.

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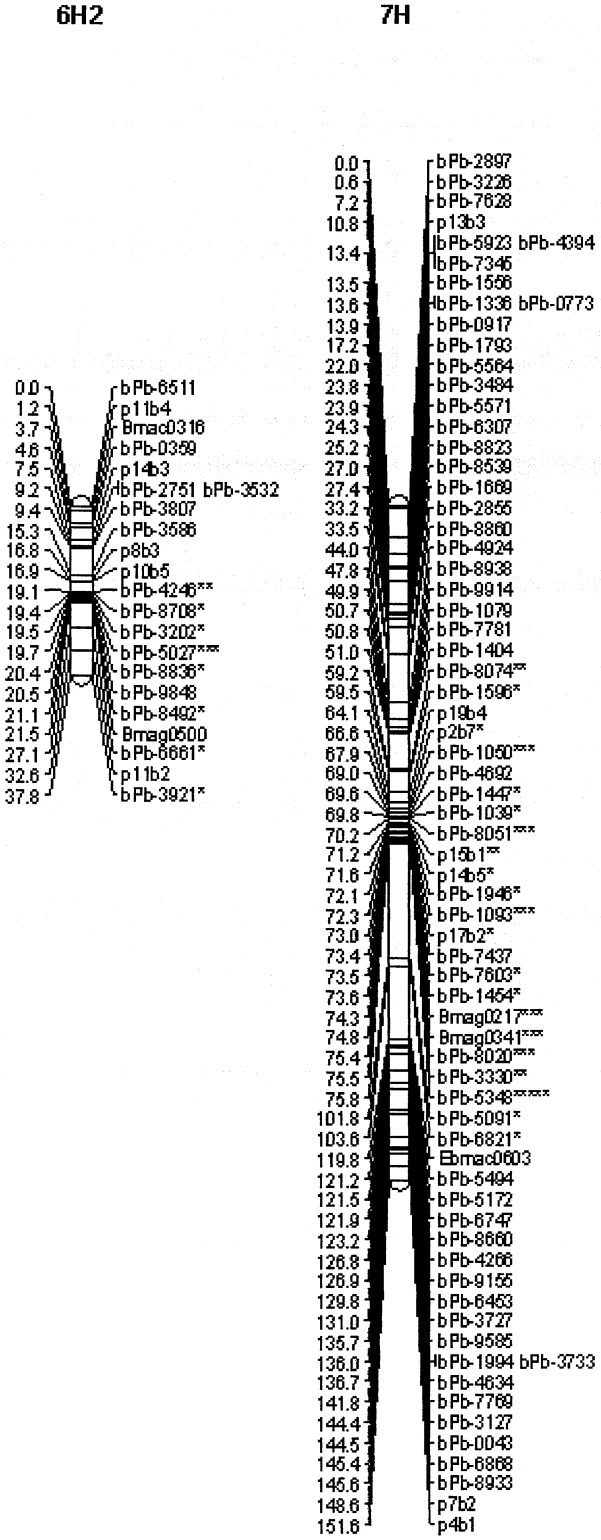
32.3 bPb-1408**
34.1 bPb-6646*
34.3 bPb-8437* bPb-6404**
40.0 bPb-4333***
40.1 bPb-0513**
40.2 bPb-3684**
40.3 bPb-3512*****
43.9 bPb-9504**
46.5 p14b1****
49.1 p9b4******
52.1 Bmac0310*
77.7 Ebmac0679
79.0 bPb-1325
82.2 p7b3*
83.7 bPb-6523
112.6 bPb-9668
114.4 bPb-5265
115.6 bPb-4765
116.3 p12b4
5H
0.0 bPb-8846****
10.9 p13b2
16.2 bPb-7360 bPb-7292
bPb-9244 bPb-4725
16.3 bPb-5766
18.9 bPb-4479
21.4 bPb-9660 bPb-2689
23.3 bPb-9868
28.1 bPb-6179
29.5 bPb-1965
29.9 bPb-4595
33.0 bPb-3138
33.1 bPb-2314
33.3 bPb-5854
35.0 bPb-8684
37.6 bPb-0171
56.6 p9b7
58.3 bPb-7277
58.4 bPb-2006
58.9 bPb-5179
59.1 bPb-1494
66.2 bPb-3700
66.9 bPb-8462*
69.1 bPb-4494
71.4 bPb-3214*
74.9 bPb-2325**
75.3 bPb-7854***
78.0 p5b1
78.8 bPb-0710***
82.8 bPb-1462***
84.4 bPb-4744*****
86.0 bPb-3879***
86.5 bPb-6126***
92.8 bPb-5596**
93.3 bPb-7395**
96.8 bPb-8022
105.1 bPb-6967
111.1 bPb-8179
112.2 p2b8
116.6 bPb-5532
117.4 p18b1
131.3 bPb-0325
145.2 bPb-0846
146.0 bPb-9306
146.6 Bmac0096
146.7 Bmag0387
155.2 bPb-6363
162.8 bPb-9327*
162.9 bPb-8256*
163.3 bPb-0536***
165.8 bPb-6568
167.5 bPb-0351*
167.7 bPb-3830*
167.8 bPb-1807*
171.7 bPb-2591
172.0 bPb-4814
172.1 bPb-8027*
172.3 bPb-2872*
174.3 bPb-1909
179.0 bPb-8580***
187.1 bPb-6051
189.3 bPb-0170
189.4 bPb-6485
190.2 bPb-5317
190.4 bPb-7599
191.3 bPb-2330
6H1
0.0 bPb-5822
2.5 GMS006
4.5 Bmag0496
5.9 p16b2
bPb-4289 bPb-4627
8.8 bPb-6164 bPb-8969*
bPb-3022
9.0 Ebmac0806
10.0 bPb-5778
10.1 bPb-5903
11.4 bPb-6121
11.9 Bmag0173
34.3 bPb-0451
35.3 p17b1
40.0 bPb-3643
46.7 bPb-0857*
52.4 bPb-5234*
52.5 bPb-6385* bPb-2062*
56.6 p9b6******
65.1 bPb-1758****
67.3 bPb-7446***
68.2 bPb-7877**
68.4 bPb-1176*
68.9 bPb-9413*
73.7 p10b1**
76.1 bPb-8054*
76.2 bPb-3760
76.3 bPb-6875
76.4 bPb-6677 bPb-2863
76.9 bPb-2940
77.4 bPb-8382
81.7 bPb-8735 bPb-3919
82.5 bPb-9817 bPb-2410
82.6 bPb-9890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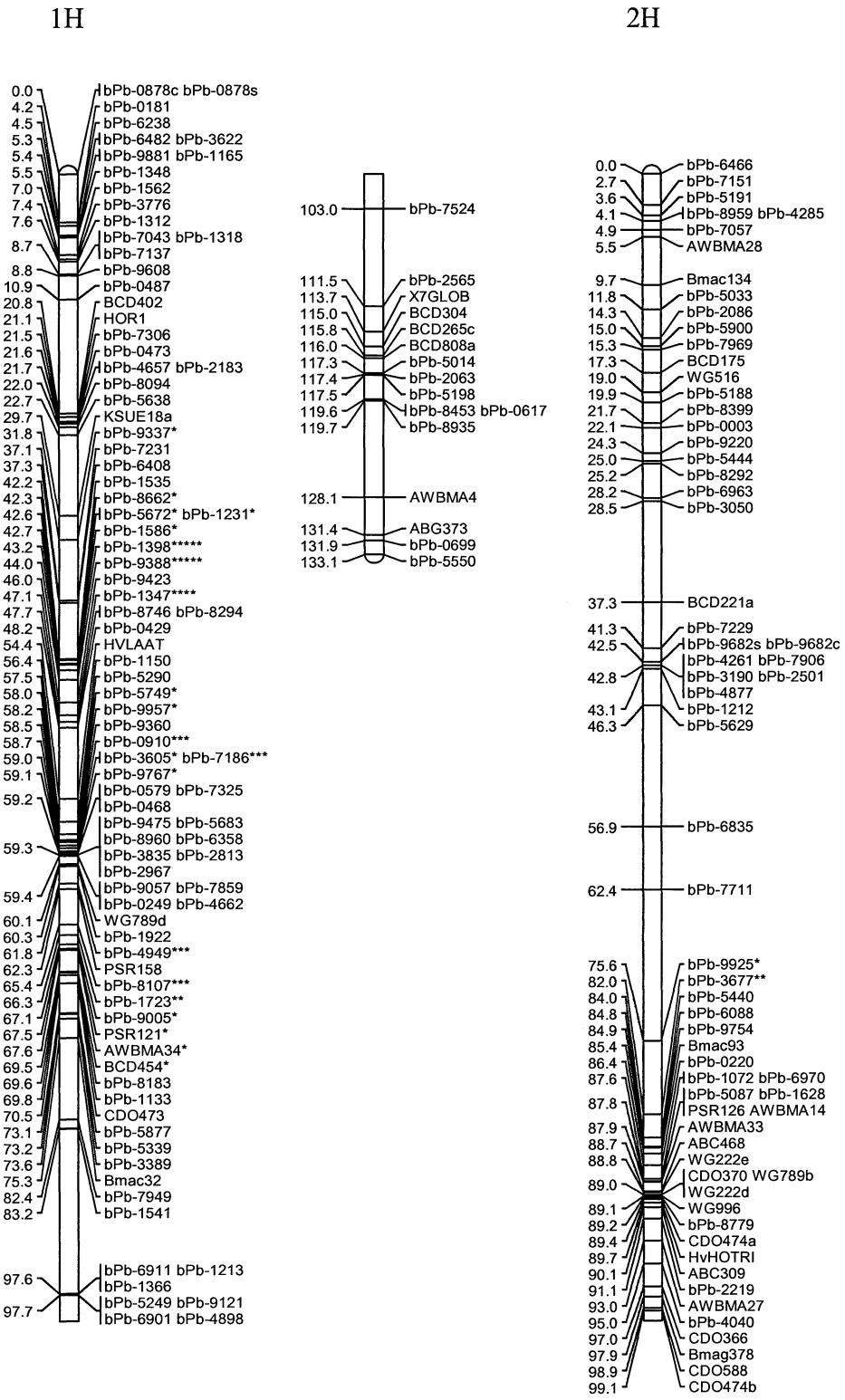
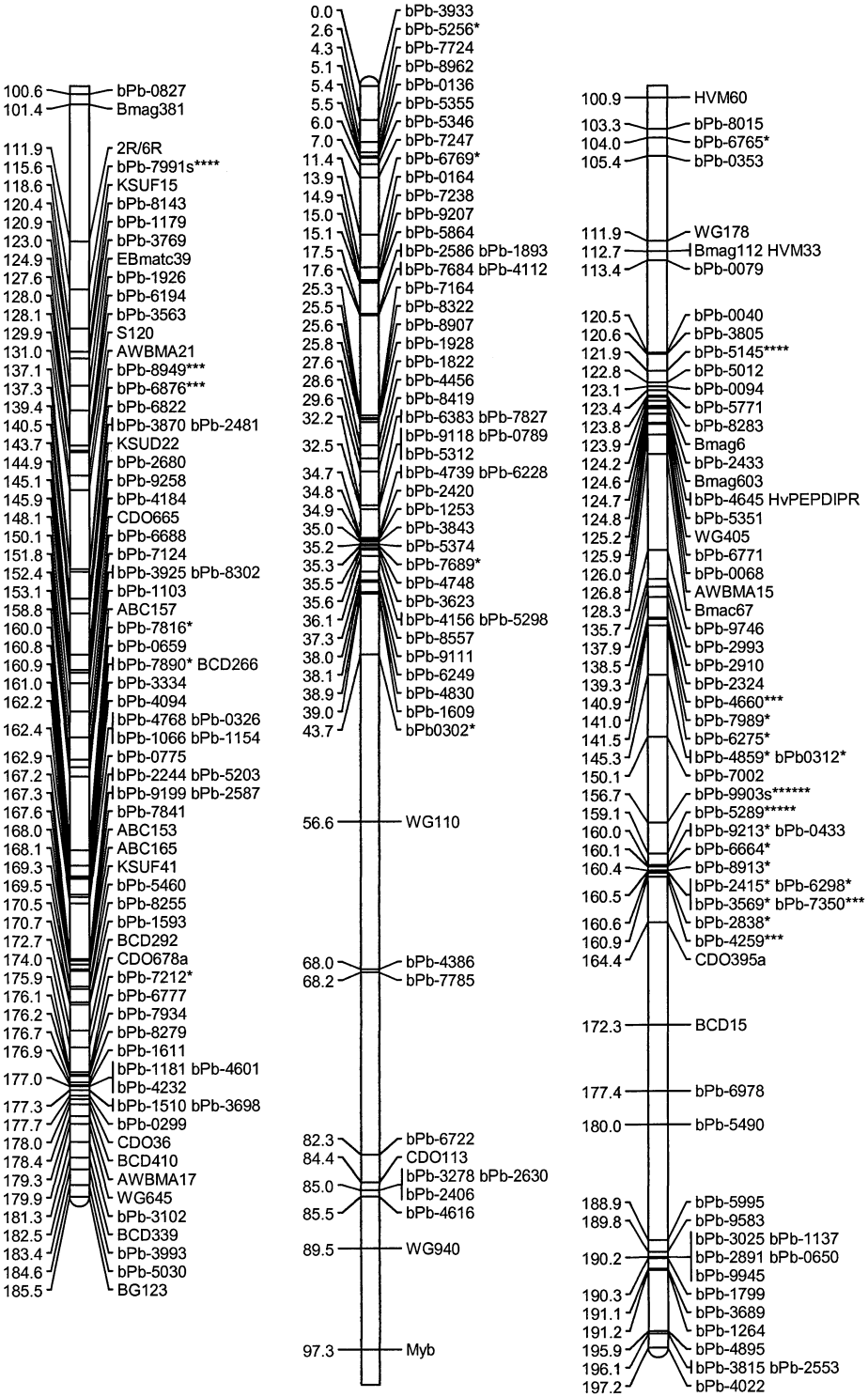
1.2 p11b4
3.7 Bmac0316
4.6 bPb-0399
7.5 p14b3
9.2 bPb-2751 bPb-3532
9.4 bPb-3807
15.3 bPb-3586
16.8 p8b3
16.9 p10b5
19.1 bPb-4246**
19.4 bPb-8708*
19.5 bPb-3202*
19.7 bPb-5027***
20.4 bPb-8836*
20.5 bPb-9848
21.1 bPb-8492*
21.5 Bmag0500
27.1 bPb-6661*
32.6 p11b2
37.8 bPb-3921*
7H
0.0 bPb-2897
0.6 bPb-3226
7.2 bPb-7628
10.8 p13b3
13.4 bPb-5923 bPb-4394
bPb-7345
13.5 bPb-1556
13.6 bPb-1336 bPb-0773
13.9 bPb-0917
17.2 bPb-1793
22.0 bPb-5564
23.8 bPb-3484
23.9 bPb-5571
24.3 bPb-6307
25.2 bPb-8823
27.0 bPb-8539
27.4 bPb-1669
33.2 bPb-2855
33.5 bPb-8860
44.0 bPb-4924
47.8 bPb-8938
49.9 bPb-9914
50.7 bPb-1079
50.8 bPb-7781
51.0 bPb-1404
59.2 bPb-8074**
59.5 bPb-1596*
64.1 p19b4
66.6 p2b7*
67.9 bPb-1050***
69.0 bPb-4692
69.6 bPb-1447*
69.8 bPb-1039*
70.2 bPb-8051***
71.2 p15b1**
71.6 p14b5*
72.1 bPb-1946*
72.3 bPb-1093***
73.0 p17b2*
73.4 bPb-7437
73.5 bPb-7603*
73.6 bPb-1454*
74.3 Bmag0217***
74.8 Bmag0341***
75.4 bPb-8020***
75.5 bPb-3330**
75.8 bPb-5348*****
101.8 bPb-5091*
103.6 bPb-6821*
119.8 Ebmac0603
121.2 bPb-5494
121.5 bPb-5172
121.9 bPb-6747
123.2 bPb-8660
126.8 bPb-4266
126.9 bPb-9155
129.8 bPb-6463
131.0 bPb-3727
135.7 bPb-9585
136.0 bPb-1994 bPb-3733
136.7 bPb-4634
141.8 bPb-7769
144.4 bPb-3127
144.5 bPb-0043
145.4 bPb-6868
145.6 bPb-8933
148.6 p7b2
151.6 p4b1

Appendix 4. The linkage map of the Clipper/Sahara population, showing the markers with segregation distortion.



3H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101.4 Bmag381
111.9 2R/6R
115.6 bPb-7991s****
118.6 KSUF15
120.4 bPb-8143
120.9 bPb-1179
123.0 bPb-3769
124.9 EBmatc39
127.6 bPb-1926
128.0 bPb-6194
128.1 bPb-3563
129.9 S120
131.0 AWBMA21
137.1 bPb-8949***
137.3 bPb-6876***
139.4 bPb-6822
140.5 bPb-3870 bPb-2481
143.7 KSUD22
144.9 bPb-2680
145.1 bPb-9258
145.9 bPb-4184
148.1 CDO665
150.1 bPb-6688
151.8 bPb-7124
152.4 bPb-3925 bPb-8302
153.1 bPb-1103
158.8 ABC157
160.0 bPb-7816*
160.8 bPb-0659
160.9 bPb-7890* BCD266
161.0 bPb-3334
162.2 bPb-4094
162.4 bPb-4768 bPb-0326
bPb-1066 bPb-1154
162.9 bPb-0775
167.2 bPb-2244 bPb-5203
167.3 bPb-9199 bPb-2587
167.6 bPb-7841
168.0 ABC153
168.1 ABC165
169.3 KSUF41
169.5 bPb-5460
170.5 bPb-8255
170.7 bPb-1593
172.7 BCD292
174.0 CDO678a
175.9 bPb-7212*
176.1 bPb-6777
176.2 bPb-7934
176.7 bPb-8279
176.9 bPb-1611
177.0 bPb-1181 bPb-4601
bPb-4232
177.3 bPb-1510 bPb-3698
177.7 bPb-0299
178.0 CDO36
178.4 BCD410
179.3 AWBMA17
179.9 WG645
181.3 bPb-3102
182.5 BCD339
183.4 bPb-3993
184.6 bPb-5030
185.5 BG123
0.0 bPb-3933
2.6 bPb-5256*
4.3 bPb-7724
5.1 bPb-8962
5.4 bPb-0136
5.5 bPb-5355
6.0 bPb-5346
7.0 bPb-7247
11.4 bPb-6769*
13.9 bPb-0164
14.9 bPb-7238
15.0 bPb-9207
15.1 bPb-5864
17.5 bPb-2586 bPb-1893
17.6 bPb-7684 bPb-4112
25.3 bPb-7164
25.5 bPb-8322
25.6 bPb-8907
25.8 bPb-1928
27.6 bPb-1822
28.6 bPb-4456
29.6 bPb-8419
32.2 bPb-6383 bPb-7827
bPb-9118 bPb-0789
32.5 bPb-5312
34.7 bPb-4739 bPb-6228
34.8 bPb-2420
34.9 bPb-1253
35.0 bPb-3843
35.2 bPb-5374
35.3 bPb-7689*
35.5 bPb-4748
35.6 bPb-3623
36.1 bPb-4156 bPb-5298
37.3 bPb-8557
38.0 bPb-9111
38.1 bPb-6249
38.9 bPb-4830
39.0 bPb-1609
43.7 bPb0302*
56.6 WG110
68.0 bPb-4386
68.2 bPb-7785
82.3 bPb-6722
84.4 CDO113
85.0 bPb-3278 bPb-2630
bPb-2406
85.5 bPb-4616
89.5 WG940
97.3 Myb
100.9 HVM60
103.3 bPb-8015
104.0 bPb-6765*
105.4 bPb-0353
111.9 WG178
112.7 Bmag112 HVM33
113.4 bPb-0079
120.5 bPb-0040
120.6 bPb-3805
121.9 bPb-5145****
122.8 bPb-5012
123.1 bPb-0094
123.4 bPb-5771
123.8 bPb-8283
123.9 Bmag6
124.2 bPb-2433
124.6 Bmag603
124.7 bPb-4645 HvPEPDIPR
124.8 bPb-5351
125.2 WG405
125.9 bPb-6771
126.0 bPb-0068
126.8 AWBMA15
128.3 Bmac67
135.7 bPb-9746
137.9 bPb-2993
138.5 bPb-2910
139.3 bPb-2324
140.9 bPb-4660***
141.0 bPb-7989*
141.5 bPb-6275*
145.3 bPb-4859* bPb0312*
150.1 bPb-7002
156.7 bPb-9903s******
159.1 bPb-5289*****
160.0 bPb-9213* bPb-0433
160.1 bPb-6664*
160.4 bPb-8913*
160.5 bPb-2415* bPb-6298*
bPb-3569* bPb-7350***
160.6 bPb-2838*
160.9 bPb-4259***
164.4 CDO395a
172.3 BCD15
177.4 bPb-6978
180.0 bPb-5490
188.9 bPb-5995
189.8 bPb-9583
190.2 bPb-3025 bPb-1137
bPb-2891 bPb-0650
bPb-9945
190.3 bPb-1799
191.1 bPb-3689
191.2 bPb-1264
195.9 bPb-4895
196.1 bPb-3815 bPb-2553
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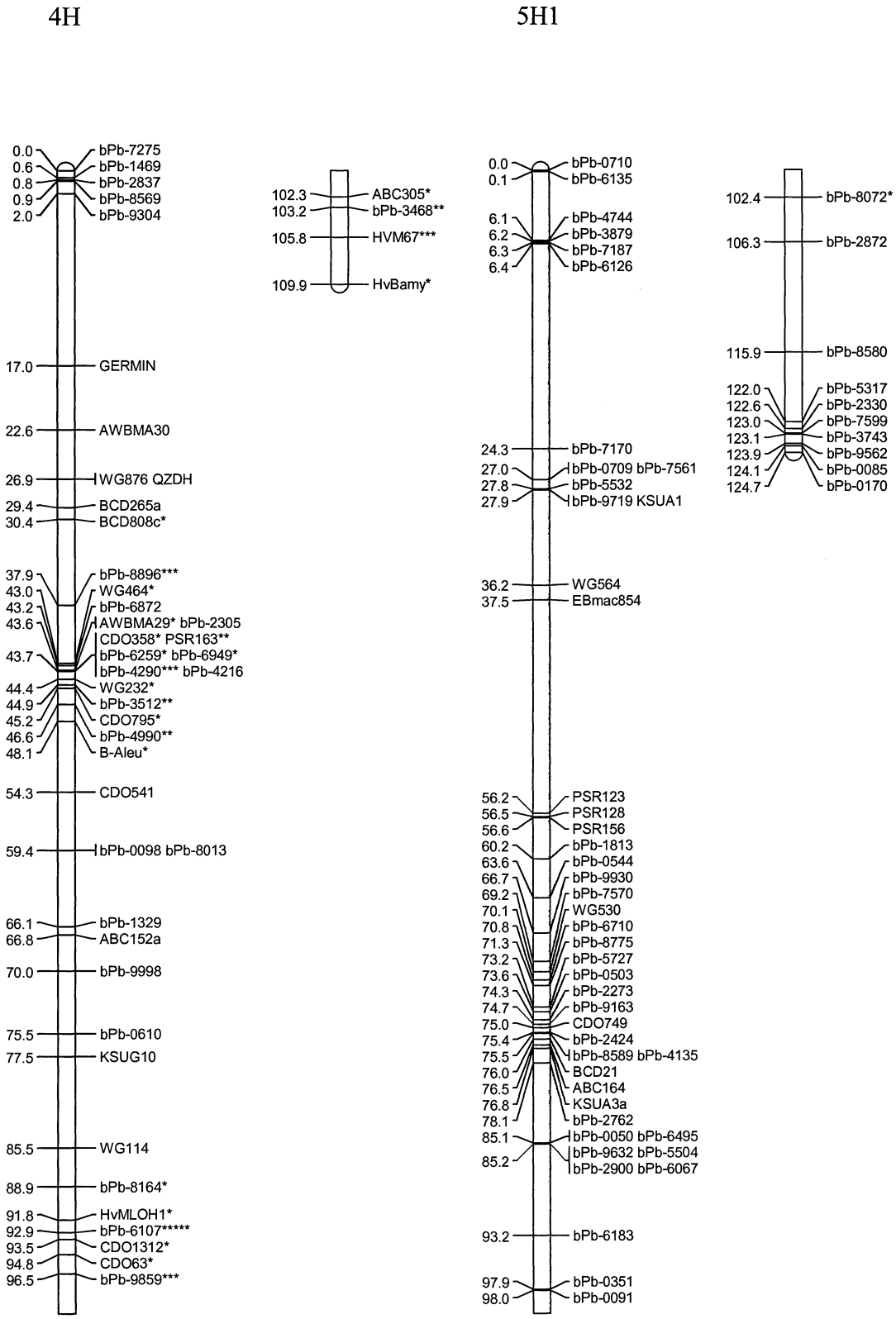
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2.0 bPb-9304
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22.6 AWBMA30
26.9 WG876 QZDH
29.4 BCD265a
30.4 BCD808c*
37.9 bPb-8896***
43.0 WG464*
43.2 bPb-6872
43.6 AWBMA29* bPb-2305
CDO358* PSR163**
43.7 bPb-6259* bPb-6949*
bPb-4290*** bPb-4216
44.4 WG232*
44.9 bPb-3512**
45.2 CDO795*
46.6 bPb-4990**
48.1 B-Aleu*
54.3 CDO541
59.4 bPb-0098 bPb-8013
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70.0 bPb-9998
75.5 bPb-0610
77.5 KSUG10
85.5 WG114
88.9 bPb-8164*
91.8 HvMLOH1*
92.9 bPb-6107*****
93.5 CDO1312*
94.8 CDO63*
96.5 bPb-9859***
102.3 ABC305*
103.2 bPb-3468**
105.8 HVM67***
109.9 HvBamy*
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0.1 bPb-6135
6.1 bPb-4744
6.2 bPb-3879
6.3 bPb-7187
6.4 bPb-6126
24.3 bPb-7170
27.0 bPb-0709 bPb-7561
27.8 bPb-5532
27.9 bPb-9719 KSUA1
36.2 WG564
37.5 EBmac854
56.2 PSR123
56.5 PSR128
56.6 PSR156
60.2 bPb-1813
63.6 bPb-0544
66.7 bPb-9930
69.2 bPb-7570
70.1 WG530
70.8 bPb-6710
71.3 bPb-8775
73.2 bPb-5727
73.6 bPb-0503
74.3 bPb-2273
74.7 bPb-9163
75.0 CDO749
75.4 bPb-2424
75.5 bPb-8589 bPb-4135
76.0 BCD21
76.5 ABC164
76.8 KSUA3a
78.1 bPb-2762
85.1 bPb-0050 bPb-6495
85.2 bPb-9632 bPb-5504
bPb-2900 bPb-6067
93.2 bPb-6183
97.9 bPb-0351
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102.4 bPb-8072*
106.3 bPb-2872
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122.0 bPb-5317
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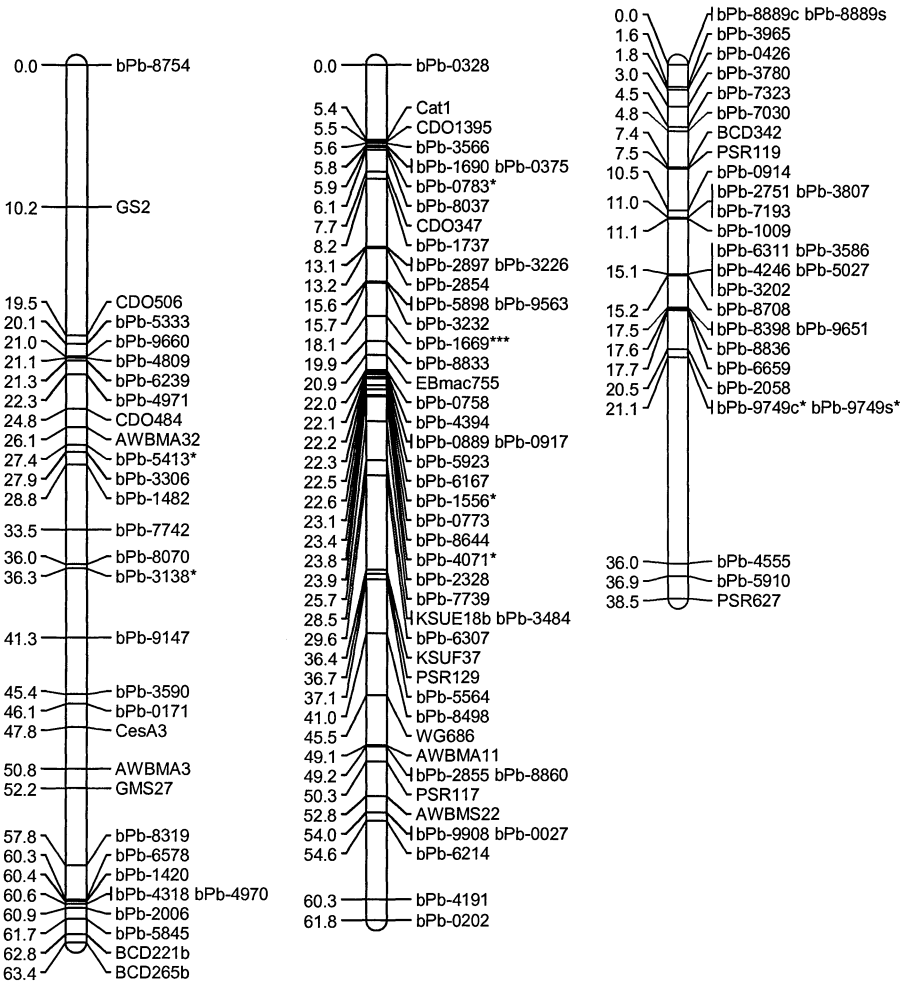
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27.9 bPb-3306
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36.3 bPb-3138*
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46.1 bPb-0171
47.8 CesA3
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60.6 bPb-4318 bPb-4970
60.9 bPb-2006
61.7 bPb-5845
62.8 BCD221b
63.4 BCD265b
0.0 bPb-0328
5.4 Cat1
5.5 CDO1395
5.6 bPb-3566
5.8 bPb-1690 bPb-0375
5.9 bPb-0783*
6.1 bPb-8037
7.7 CDO347
8.2 bPb-1737
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23.1 bPb-0773
23.4 bPb-8644
23.8 bPb-4071*
23.9 bPb-2328
25.7 bPb-7739
28.5 KSUE18b bPb-3484
29.6 bPb-6307
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36.7 PSR129
37.1 bPb-5564
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45.5 WG686
49.1 AWBMA11
49.2 bPb-2855 bPb-8860
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52.8 AWBMS22
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54.6 bPb-6214
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61.8 bPb-0202
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1.8 bPb-0426
3.0 bPb-3780
4.5 bPb-7323
4.8 bPb-7030
7.4 BCD342
7.5 PSR119
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11.1 bPb-7193
bPb-1009
15.1 bPb-6311 bPb-3586
bPb-4246 bPb-5027
bPb-3202
15.2 bPb-8708
17.5 bPb-8398 bPb-9651
17.6 bPb-8836
17.7 bPb-6659
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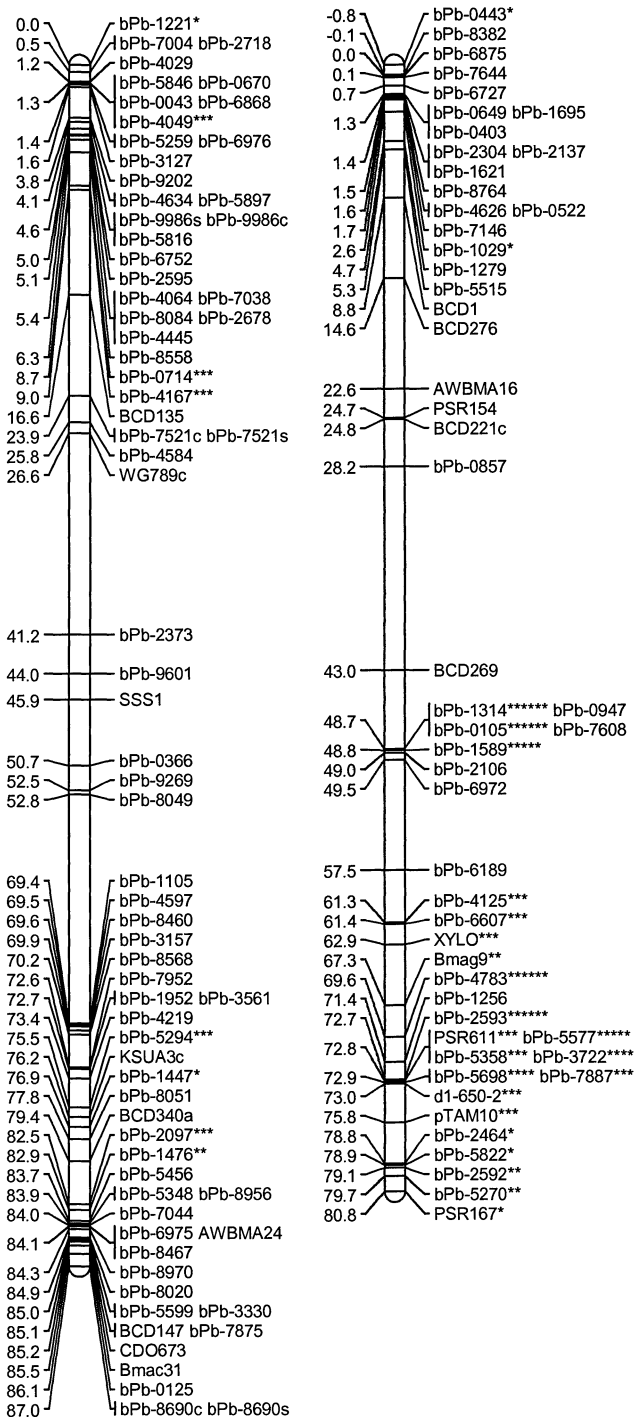
21.1 bPb-9749c* bPb-9749s*
36.0 bPb-4555
36.9 bPb-5910
38.5 PSR627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bPb-4049***
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1.6 bPb-3127
3.8 bPb-9202
4.1 bPb-4634 bPb-5897
4.6 bPb-9986s bPb-9986c
bPb-5816
5.0 bPb-6752
5.1 bPb-2595
5.4 bPb-4064 bPb-7038
bPb-8084 bPb-2678
bPb-4445
6.3 bPb-8558
8.7 bPb-0714***
9.0 bPb-4167***
16.6 BCD135
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25.8 bPb-4584
26.6 WG789c
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52.5 bPb-9269
52.8 bPb-8049
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69.5 bPb-4597
69.6 bPb-8460
69.9 bPb-3157
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73.4 bPb-4219
75.5 bPb-5294***
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76.9 bPb-1447*
77.8 bPb-8051
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82.9 bPb-1476**
83.7 bPb-5456
83.9 bPb-5348 bPb-8956
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bPb-8467
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84.9 bPb-8020
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85.1 BCD147 bPb-7875
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bPb-0403
1.4 bPb-2304 bPb-2137
bPb-1621
1.5 bPb-8764
1.6 bPb-4626 bPb-0522
1.7 bPb-7146
2.6 bPb-1029*
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5.3 bPb-5515
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14.6 BCD276
22.6 AWBMA16
24.7 PSR154
24.8 BCD221c
28.2 bPb-0857
43.0 BCD269
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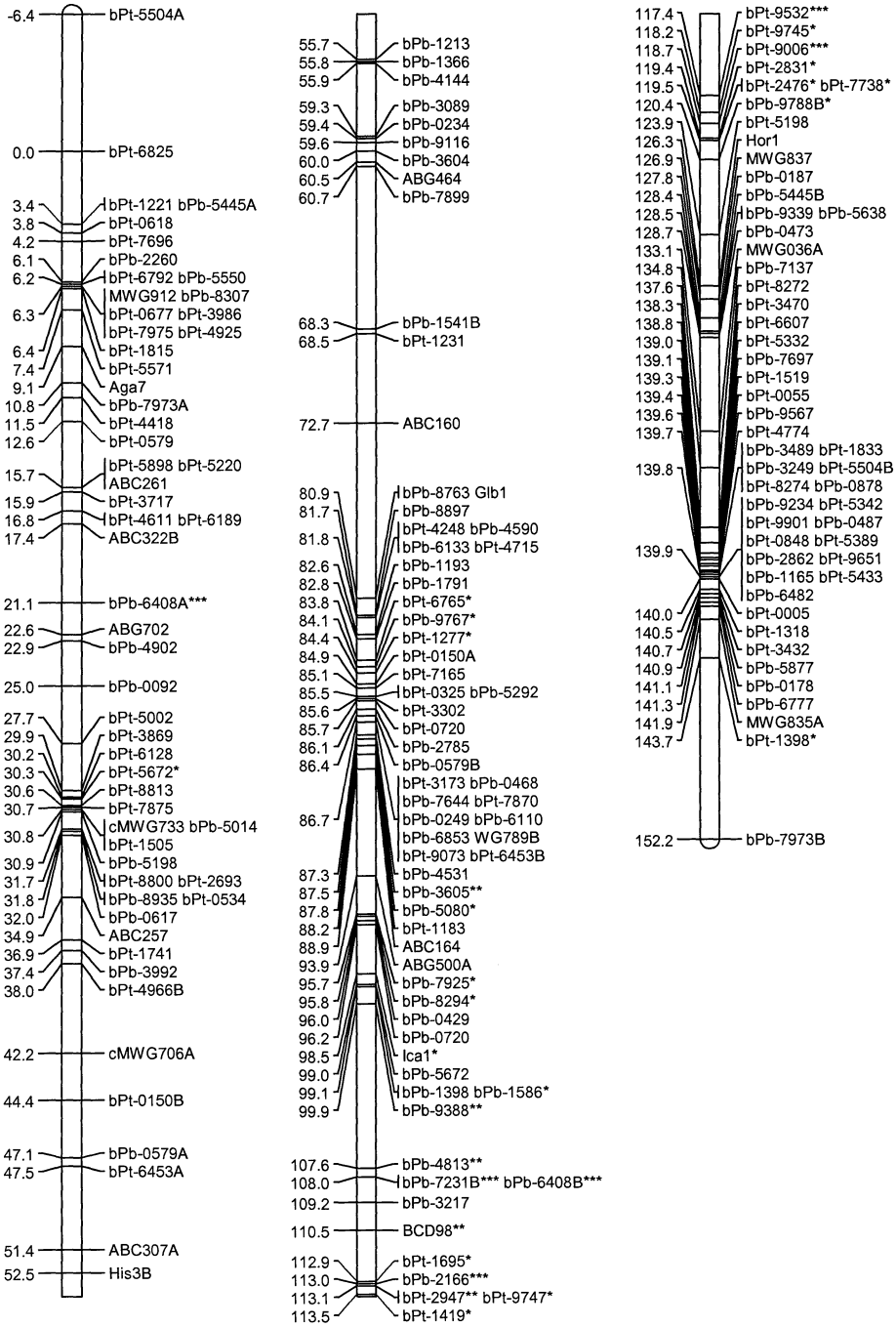
bPb-0105****** bPb-7608
48.8 bPb-1589*****
49.0 bPb-2106
49.5 bPb-6972
57.5 bPb-6189
61.3 bPb-4125***
61.4 bPb-6607***
62.9 XYLO***
67.3 Bmag9**
69.6 bPb-4783******
71.4 bPb-1256
72.7 bPb-2593******
72.8 PSR611*** bPb-5577*****
bPb-5358*** bPb-3722*****
72.9 bPb-5698***** bPb-7887***
73.0 d1-650-2***
75.8 pTAM10***
78.8 bPb-2464*
78.9 bPb-5822*
79.1 bPb-2592**
79.7 bPb-5270**
80.8 PSR167*

Appendix 5. The linkage map of the Steptoe/Morex population, showing the markers with segregation distortion.

1H



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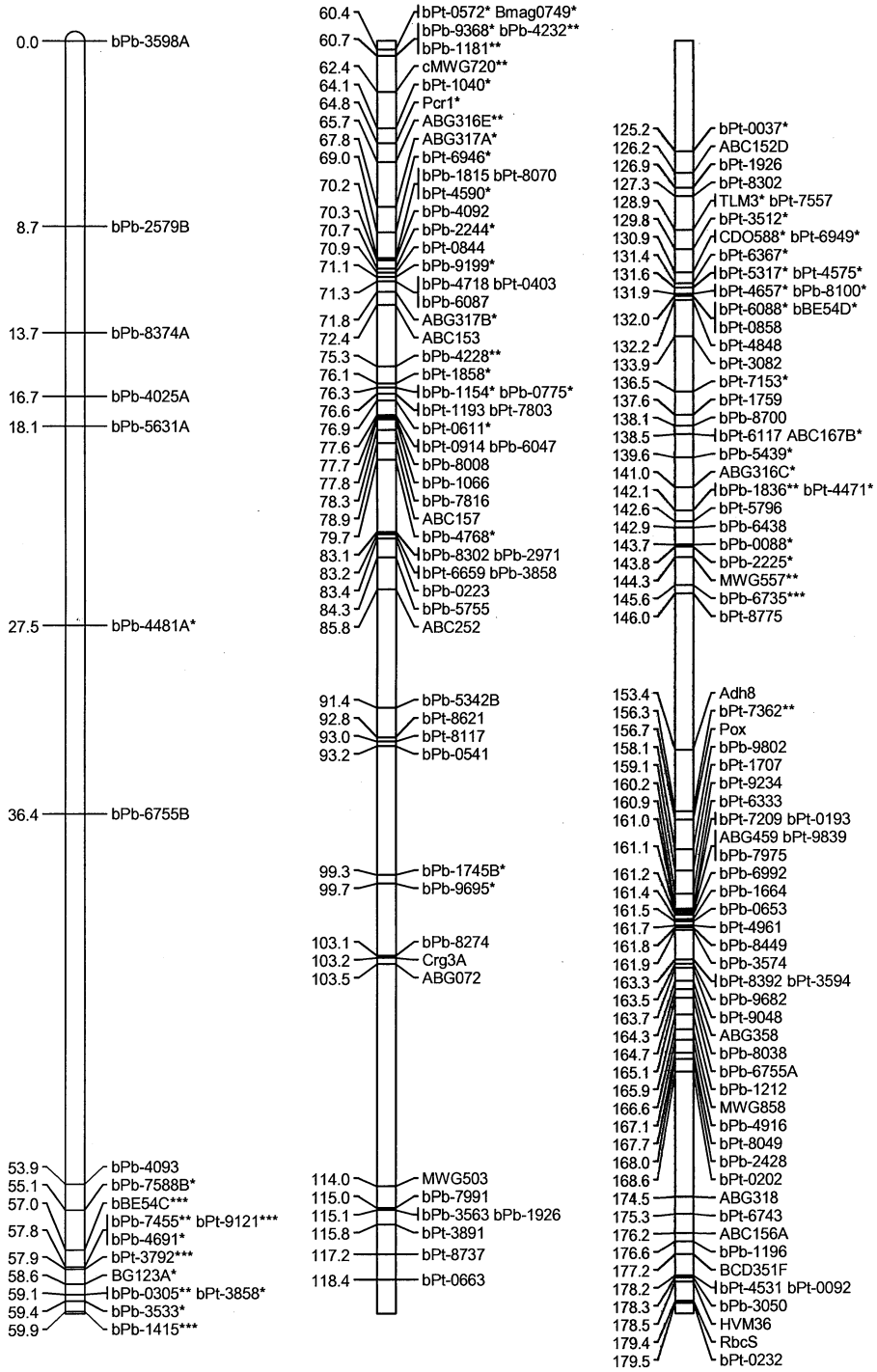
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27.5 bPb-4481A*
36.4 bPb-6755B
53.9 bPb-4093
55.1 bPb-7588B*
57.0 bBE54C***
57.8 bPb-7455** bPt-9121***
bPb-4691*
57.9 bPt-3792***
58.6 BG123A*
59.1 bPb-0305** bPt-3858*
59.4 bPb-3533*
59.9 bPb-1415***
60.4 bPt-0572* Bmag0749*
60.7 bPb-9368* bPb-4232**
bPb-1181**
62.4 cMWG720**
64.1 bPt-1040*
64.8 Pcr1*
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67.8 ABG317A*
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bPt-4590*
70.3 bPb-4092
70.7 bPb-2244*
70.9 bPt-0844
71.1 bPb-9199*
71.3 bPb-4718 bPt-0403
bPb-6087
71.8 ABG317B*
72.4 ABC153
75.3 bPb-4228**
76.1 bPt-1858*
76.3 bPb-1154* bPb-0775*
76.6 bPt-1193 bPt-7803
76.9 bPt-0611*
77.6 bPt-0914 bPb-6047
77.7 bPb-8008
77.8 bPb-1066
78.3 bPb-7816
78.9 ABC157
79.7 bPb-4768*
83.1 bPb-8302 bPb-2971
83.2 bPt-6659 bPb-3858
83.4 bPb-0223
84.3 bPb-5755
85.8 ABC252
91.4 bPb-5342B
92.8 bPt-8621
93.0 bPt-8117
93.2 bPb-0541
99.3 bPb-1745B*
99.7 bPb-9695*
103.1 bPb-8274
103.2 Crg3A
103.5 ABG072
114.0 MWG503
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115.1 bPb-3563 bPb-1926
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118.4 bPt-0663
125.2 bPt-0037*
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126.9 bPt-1926
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128.9 TLM3* bPt-7557
129.8 bPt-3512*
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143.8 bPb-2225*
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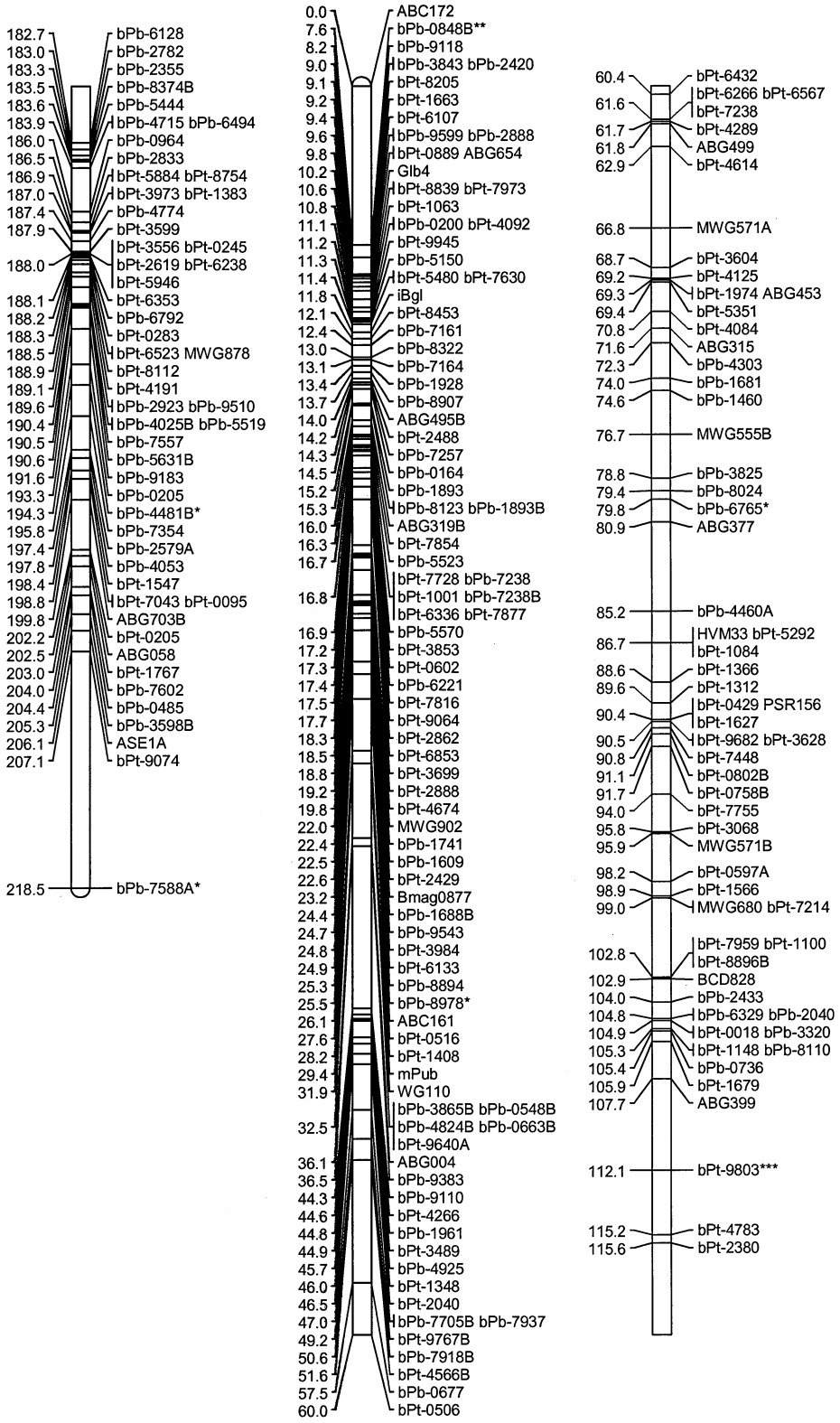
3H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bPt-5946
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190.6 bPb-5631B
191.6 bPb-9183
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194.3 bPb-4481B*
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197.8 bPb-4053
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24.9 bPt-6133
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25.5 bPb-8978*
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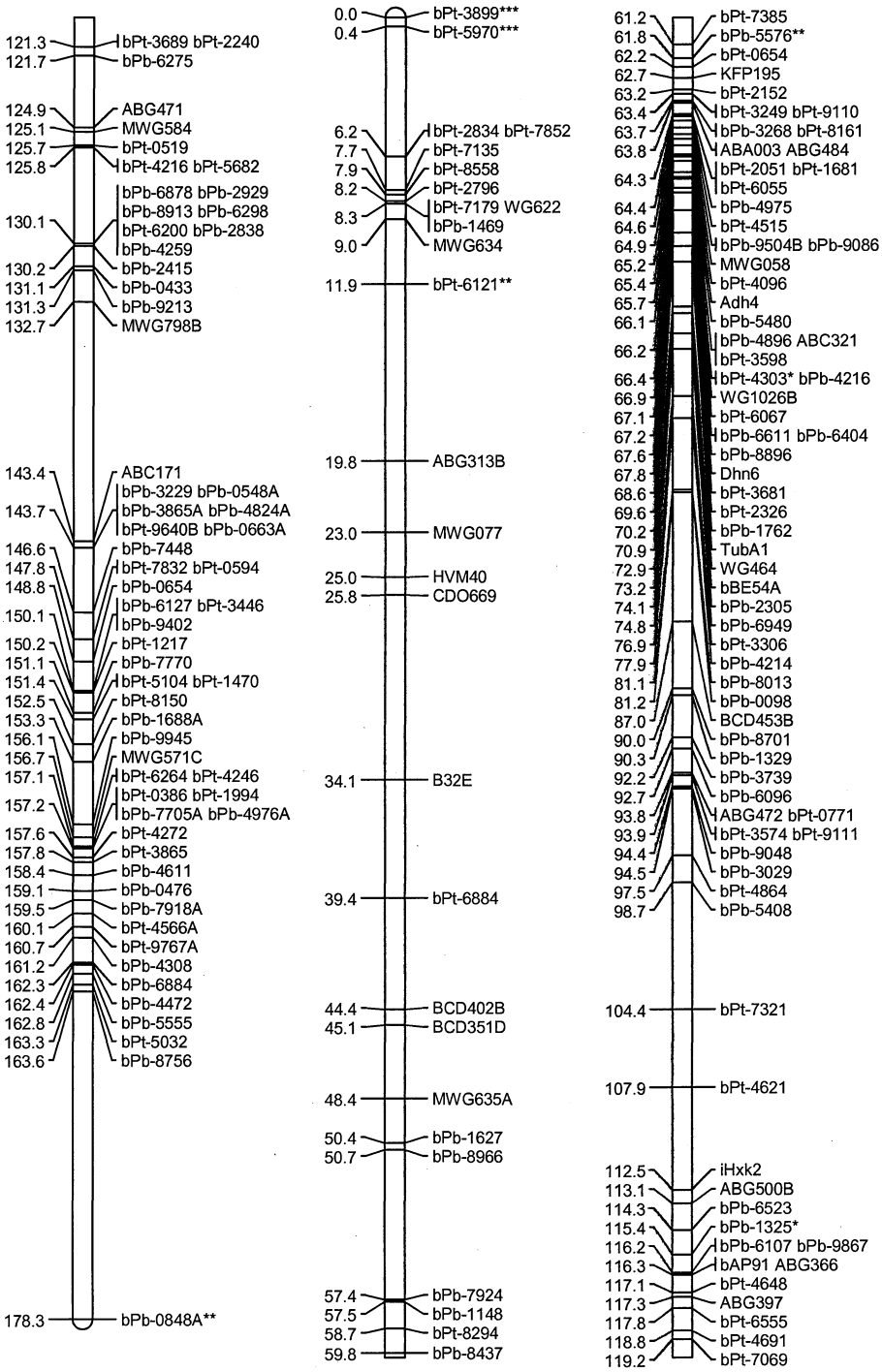
4H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7.9 bPt-8558
8.2 bPt-2796
8.3 bPt-7179 WG622 bPb-1469
9.0 MWG634
11.9 bPt-6121**
19.8 ABG313B
23.0 MWG077
25.0 HVM40
25.8 CDO669
34.1 B32E
39.4 bPt-6884
44.4 BCD402B
45.1 BCD351D
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50.7 bPb-8966
57.4 bPb-7924
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59.8 bPb-8437
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61.8 bPb-5576**
62.2 bPt-0654
62.7 KFP195
63.2 bPt-2152
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63.7 bPb-3268 bPt-8161
63.8 ABA003 ABG484
64.3 bPt-2051 bPt-1681 bPt-6055
64.4 bPb-4975
64.6 bPt-4515
64.9 bPb-9504B bPb-9086
65.2 MWG058
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65.7 Adh4
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66.2 bPb-4896 ABC321 bPt-3598
66.4 bPt-4303* bPb-4216
66.9 WG1026B
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67.2 bPb-6611 bPb-6404
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67.8 Dhn6
68.6 bPt-3681
69.6 bPt-2326
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81.1 bPb-8013
81.2 bPb-0098
87.0 BCD453B
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93.8 ABG472 bPt-0771
93.9 bPt-3574 bPt-9111
94.4 bPb-9048
94.5 bPb-3029
97.5 bPt-4864
98.7 bPb-5408
104.4 bPt-7321
107.9 bPt-4621
112.5 iHxk2
113.1 ABG500B
114.3 bPb-6523
115.4 bPb-1325*
116.2 bPb-6107 bPb-9867
116.3 bAP91 ABG366
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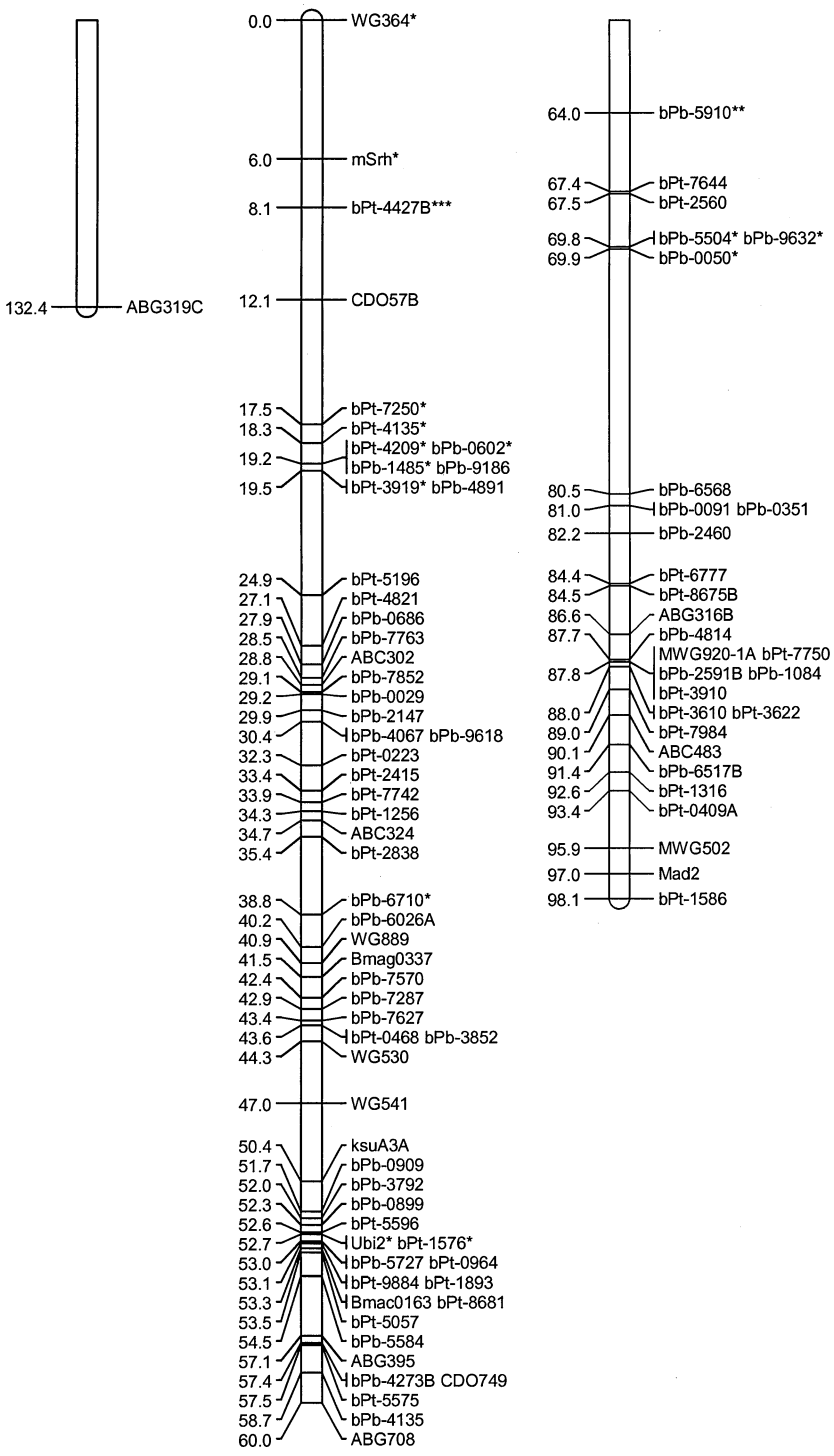
5H1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19.2 bPt-4209* bPb-0602*
bPb-1485* bPb-9186
19.5 bPt-3919* bPb-4891
24.9 bPt-5196
27.1 bPt-4821
27.9 bPb-0686
28.5 bPb-7763
28.8 ABC302
29.1 bPb-7852
29.2 bPb-0029
29.9 bPb-2147
30.4 bPb-4067 bPb-9618
32.3 bPt-0223
33.4 bPt-2415
33.9 bPt-7742
34.3 bPt-1256
34.7 ABC324
35.4 bPt-2838
38.8 bPb-6710*
40.2 bPb-6026A
40.9 WG889
41.5 Bmag0337
42.4 bPb-7570
42.9 bPb-7287
43.4 bPb-7627
43.6 bPt-0468 bPb-3852
44.3 WG530
47.0 WG541
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51.7 bPb-0909
52.0 bPb-3792
52.3 bPb-0899
52.6 bPt-5596
52.7 Ubi2* bPt-1576*
53.0 bPb-5727 bPt-0964
53.1 bPt-9884 bPt-1893
53.3 Bmac0163 bPt-8681
53.5 bPt-5057
54.5 bPb-5584
57.1 ABG395
57.4 bPb-4273B CDO749
57.5 bPt-5575
58.7 bPb-4135
60.0 ABG708
64.0 bPb-5910**
67.4 bPt-7644
67.5 bPt-2560
69.8 bPb-5504* bPb-9632*
69.9 bPb-0050*
80.5 bPb-6568
81.0 bPb-0091 bPb-0351
82.2 bPb-2460
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84.5 bPt-8675B
86.6 ABG316B
87.7 bPb-4814
87.8 MWG920-1A bPt-7750
bPb-2591B bPb-1084
bPt-3910
88.0 bPt-3610 bPt-3622
89.0 bPt-7984
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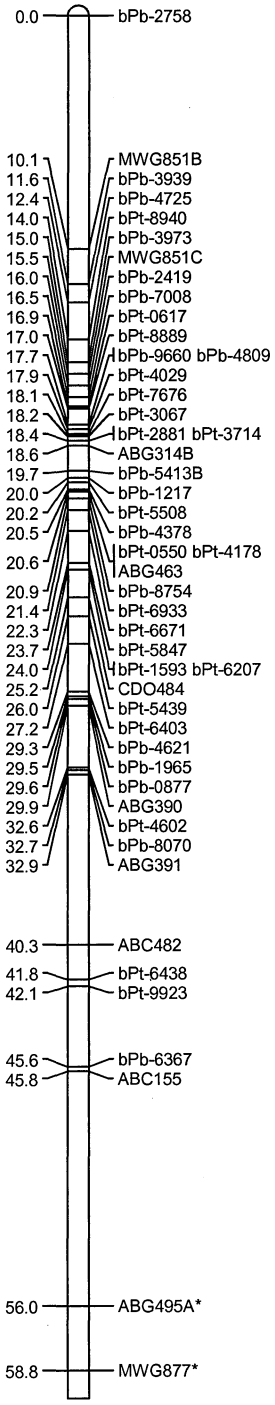
5H2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16.0 bPb-2419
16.5 bPb-7008
16.9 bPt-0617
17.0 bPt-8889
17.7 bPb-9660 bPb-4809
17.9 bPt-4029
18.1 bPt-7676
18.2 bPt-3067
18.4 bPt-2881 bPt-3714
18.6 ABG314B
19.7 bPb-5413B
20.0 bPb-1217
20.2 bPt-5508
20.5 bPb-4378
20.6 bPt-0550 bPt-4178
ABG463
20.9 bPb-8754
21.4 bPt-6933
22.3 bPt-6671
23.7 bPt-5847
24.0 bPt-1593 bPt-6207
25.2 CDO484
26.0 bPt-5439
27.2 bPt-6403
29.3 bPb-4621
29.5 bPb-1965
29.6 bPb-0877
29.9 ABG390
32.6 bPt-4602
32.7 bPb-8070
32.9 ABG391
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42.1 bPt-9923
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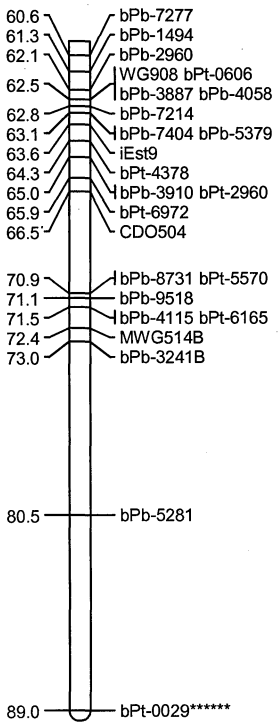
45.8 ABC155
56.0 ABG495A*
58.8 MWG877*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63.1 bPb-7404 bPb-5379
63.6 iEst9
64.3 bPt-4378
65.0 bPb-3910 bPb-2960
65.9 bPt-6972
66.5 CDO504
70.9 bPb-8731 bPt-5570
71.1 bPb-9518
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72.4 MWG514B
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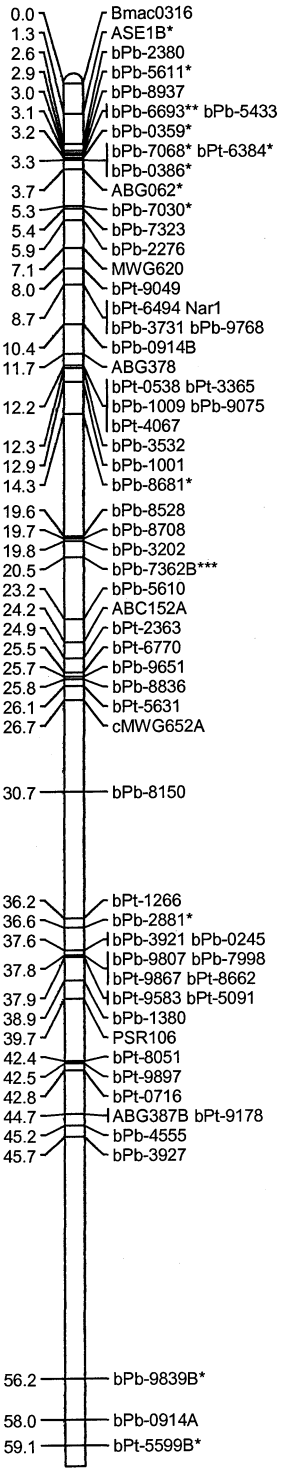
6H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bPb-7068* bPt-6384*
3.3 bPb-0386*
3.7 ABG062*
5.3 bPb-7030*
5.4 bPb-7323
5.9 bPb-2276
7.1 MWG620
8.0 bPt-9049
bPt-6494 Nar1
8.7 bPb-3731 bPb-9768
10.4 bPb-0914B
11.7 ABG378
bPt-0538 bPt-3365
12.2 bPb-1009 bPb-9075
bPt-4067
12.3 bPb-3532
12.9 bPb-1001
14.3 bPb-8681*
19.6 bPb-8528
19.7 bPb-8708
19.8 bPb-3202
20.5 bPb-7362B***
23.2 bPb-5610
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25.5 bPt-6770
25.7 bPb-9651
25.8 bPb-8836
26.1 bPt-5631
26.7 cMWG652A
30.7 bPb-8150
36.2 bPt-1266
36.6 bPb-2881*
37.6 bPb-3921 bPb-0245
bPb-9807 bPb-7998
37.8 bPt-9867 bPt-8662
37.9 bPt-9583 bPt-5091
38.9 bPb-1380
39.7 PSR106
42.4 bPt-8051
42.5 bPt-9897
42.8 bPt-0716
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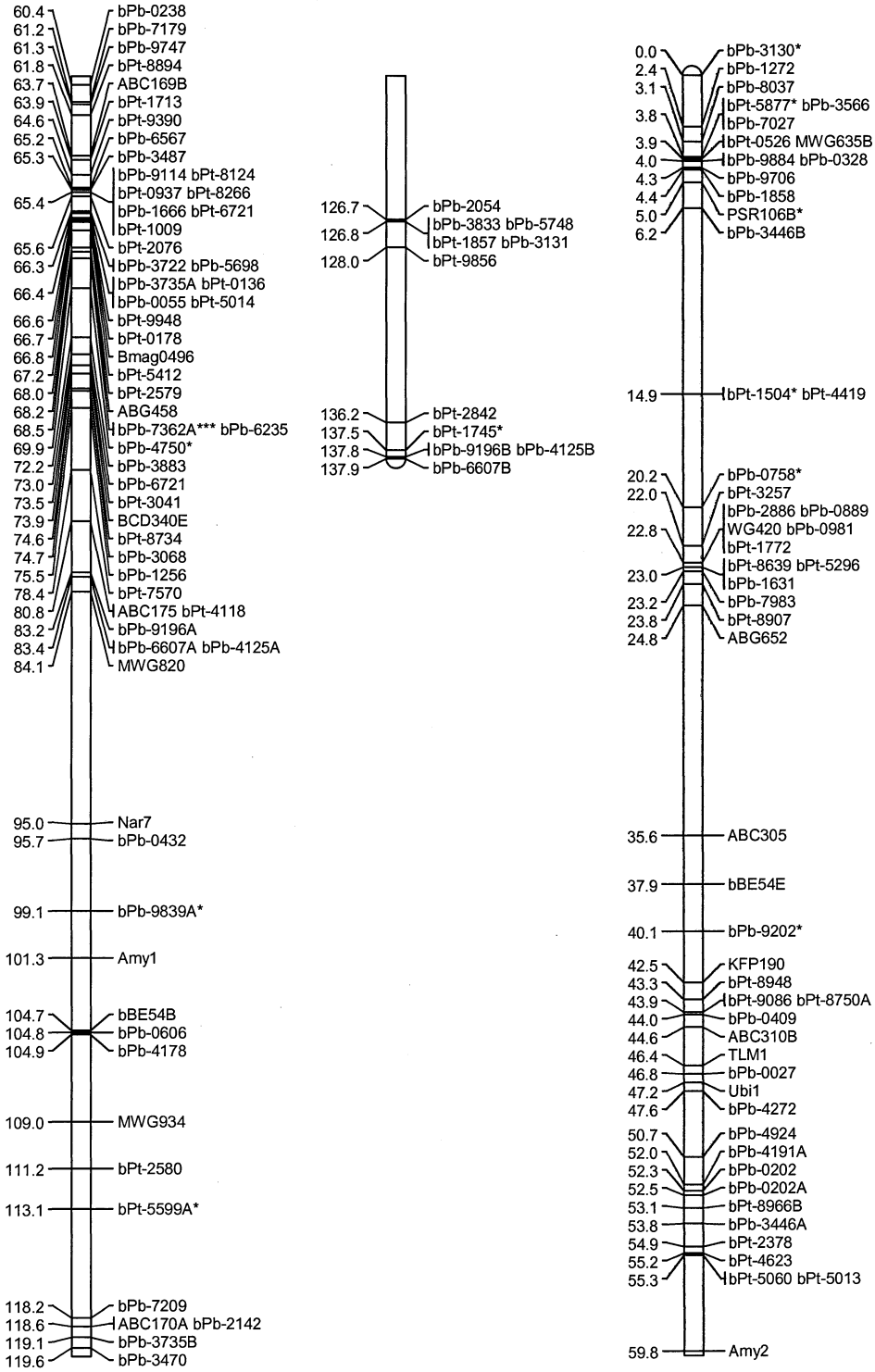
7H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bPt-1009
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bPb-0055 bPt-5014
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66.7 bPt-0178
66.8 Bmag0496
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68.5 bPb-7362A*** bPb-6235
69.9 bPb-4750*
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73.0 bPb-6721
73.5 bPt-3041
73.9 BCD340E
74.6 bPt-8734
74.7 bPb-3068
75.5 bPb-1256
78.4 bPt-7570
80.8 ABC175 bPt-4118
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3.9 bPt-0526 MWG635B
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bPt-8639 bPt-5296
23.0 bPb-1631
23.2 bPb-7983
23.8 bPt-8907
24.8 ABG652
35.6 ABC305
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40.1 bPb-9202*
42.5 KFP190
43.3 bPt-8948
43.9 bPt-9086 bPt-8750A
44.0 bPb-0409
44.6 ABC310B
46.4 TLM1
46.8 bPb-0027
47.2 Ubi1
47.6 bPb-4272
50.7 bPb-4924
52.0 bPb-4191A
52.3 bPb-0202
52.5 bPb-0202A
53.1 bPt-8966B
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54.9 bPt-2378
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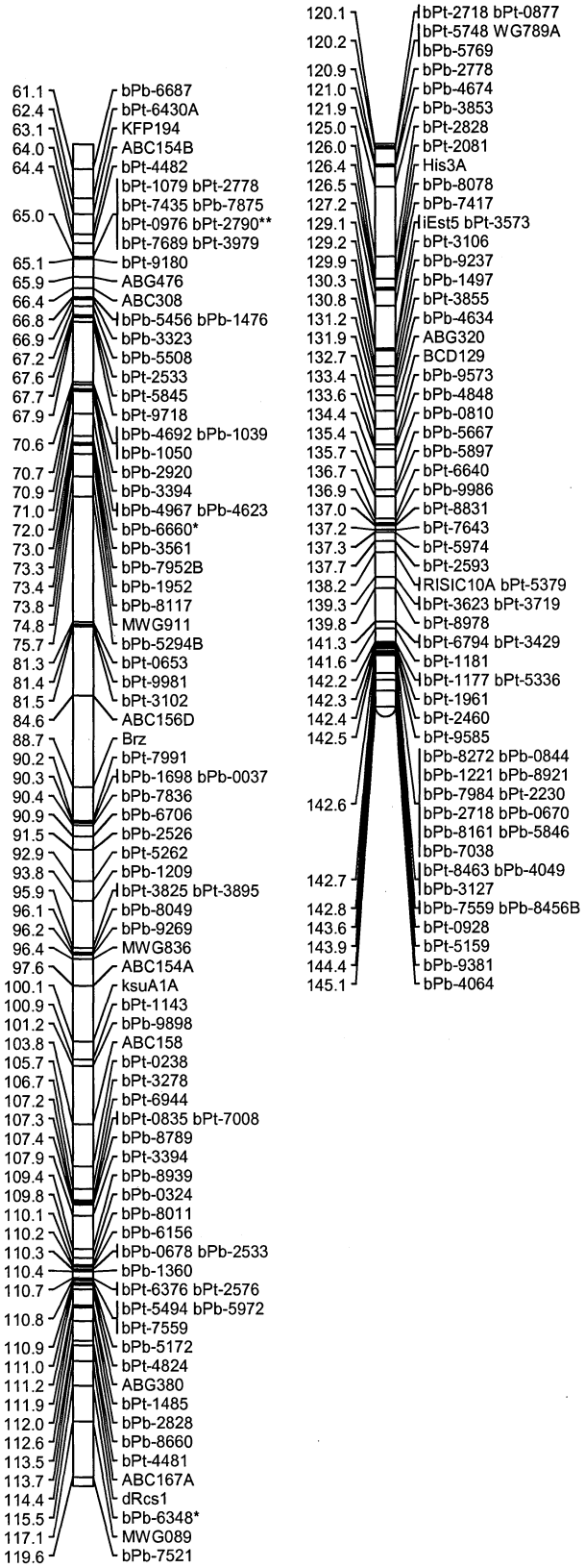
59.8 Amy2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65.0 bPt-0976 bPt-2790**
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65.1 bPt-9180
65.9 ABG476
66.4 ABC308
66.8 bPb-5456 bPb-1476
66.9 bPb-3323
67.2 bPb-5508
67.6 bPt-2533
67.7 bPt-5845
67.9 bPt-9718
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bPb-1050
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70.9 bPb-3394
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72.0 bPb-6660*
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73.3 bPb-7952B
73.4 bPb-1952
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81.4 bPt-9981
81.5 bPt-3102
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88.7 Brz
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90.4 bPb-7836
90.9 bPb-6706
91.5 bPb-2526
92.9 bPt-5262
93.8 bPb-1209
95.9 bPt-3825 bPt-3895
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101.2 bPb-9898
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bPt-7559
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115.5 bPb-6348*
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bPb-5769
120.9 bPb-2778
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139.8 bPt-8978
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141.6 bPt-1181
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bPb-7038
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bPb-3127
142.8 bPb-7559 bPb-8456B
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143.9 bPt-5159
144.4 bPb-9381
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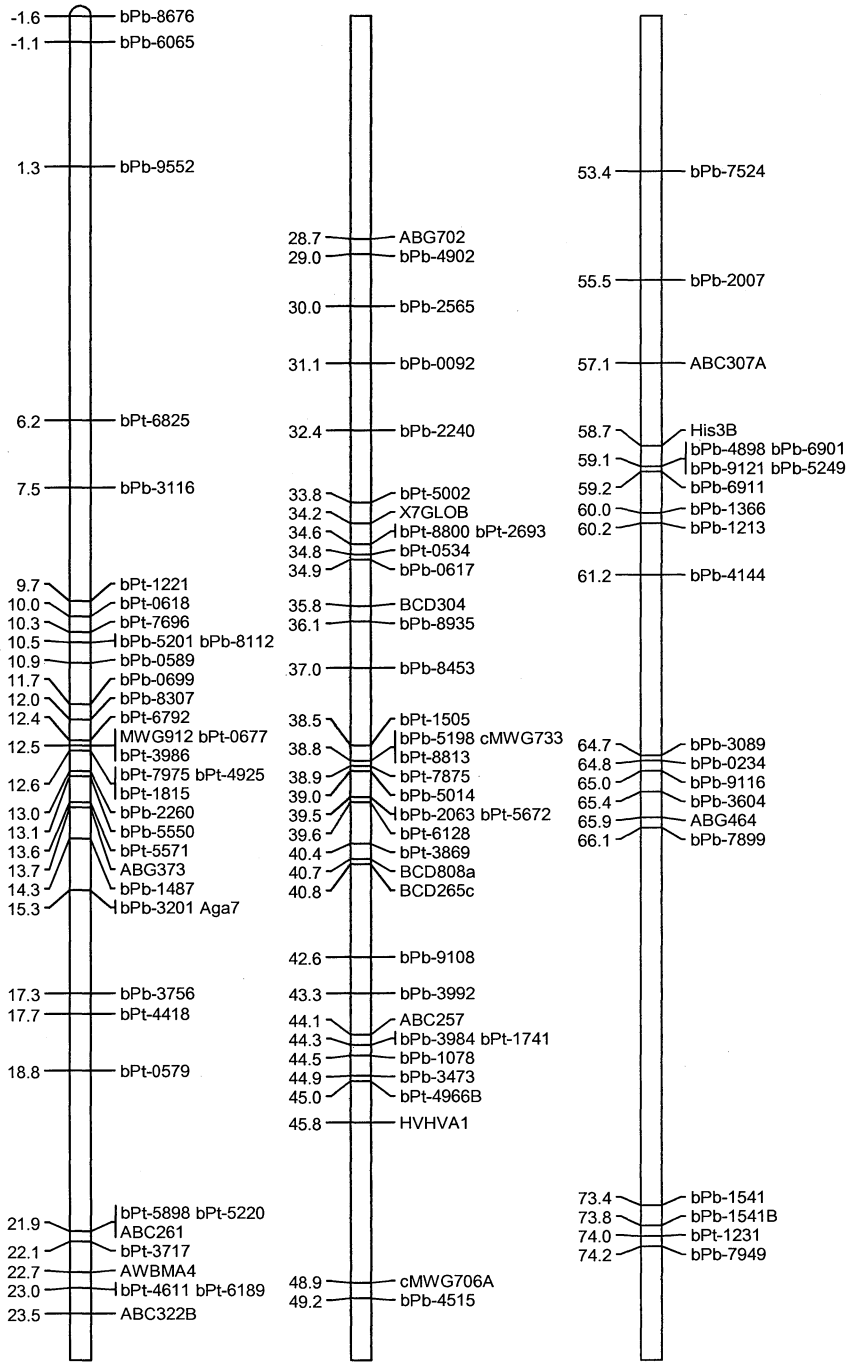
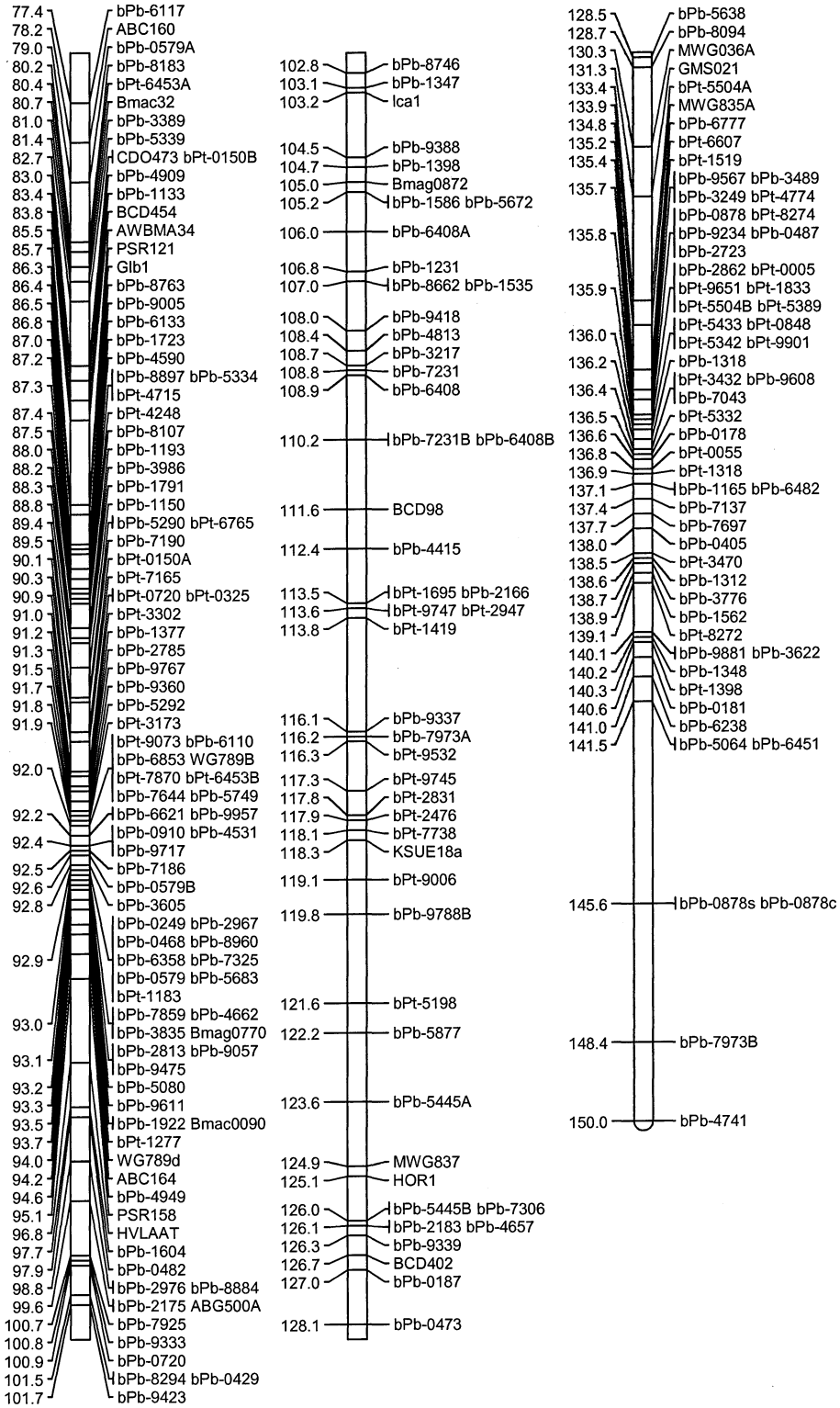
Appendix 6. A new barley consensus map based on mapping populations of Franklin/Tx9425, Franklin/Yerong, Clipper/Sahara, and Steptoe/Morex.

Chromosome1H

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82.7 CDO473 bPt-0150B
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83.8 BCD454
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87.5 bPb-8107
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97.7 bPb-1604
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126.3 bPb-9339
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128.7 bPb-8094
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133.4 bPt-5504A
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bPb-2723
bPb-2862 bPt-0005
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bPt-5504B bPt-5389
bPt-5433 bPt-0848
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136.2 bPb-1318
bPt-3432 bPb-9608
136.4 bPb-7043
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136.6 bPb-0178
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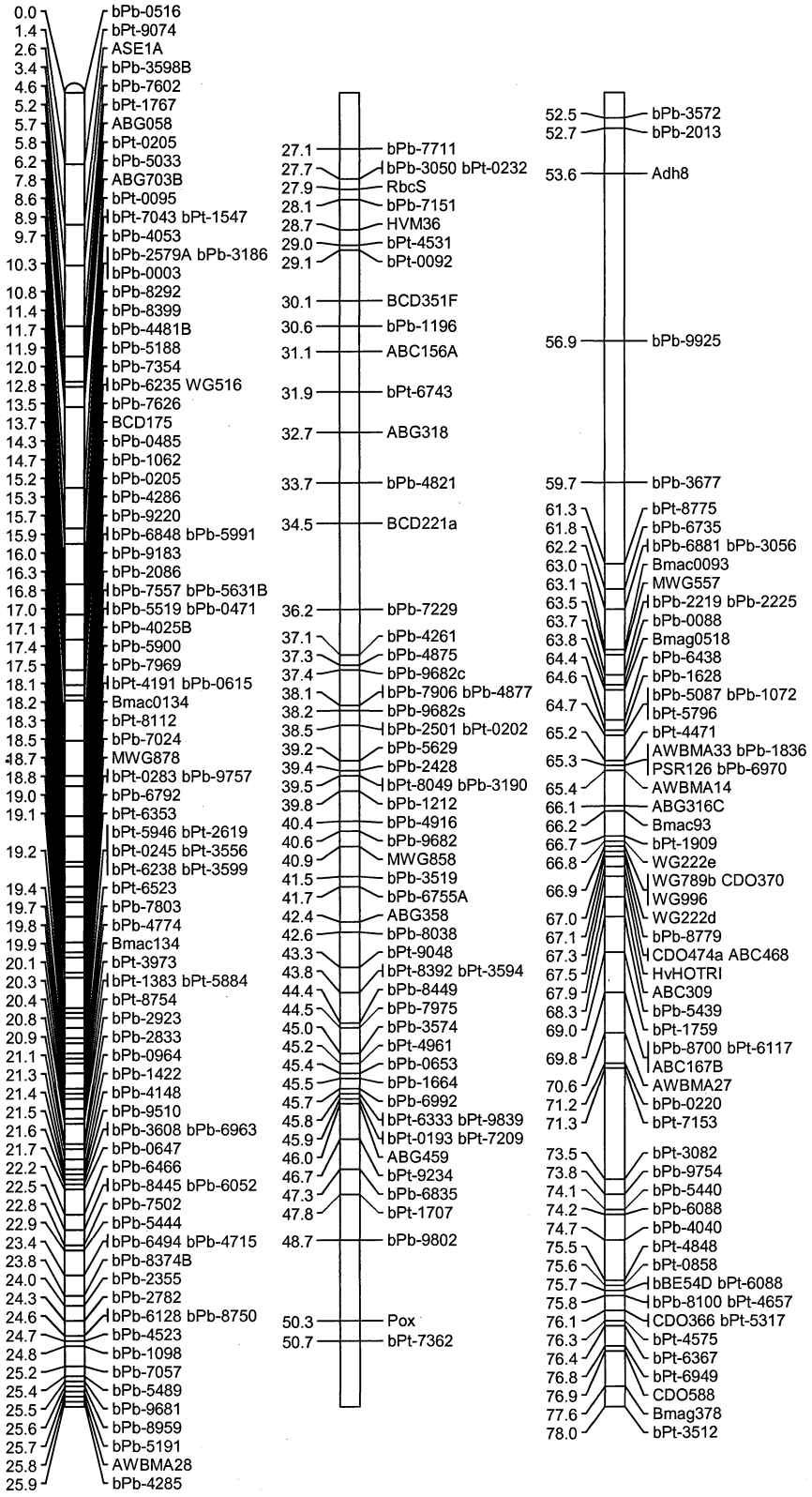
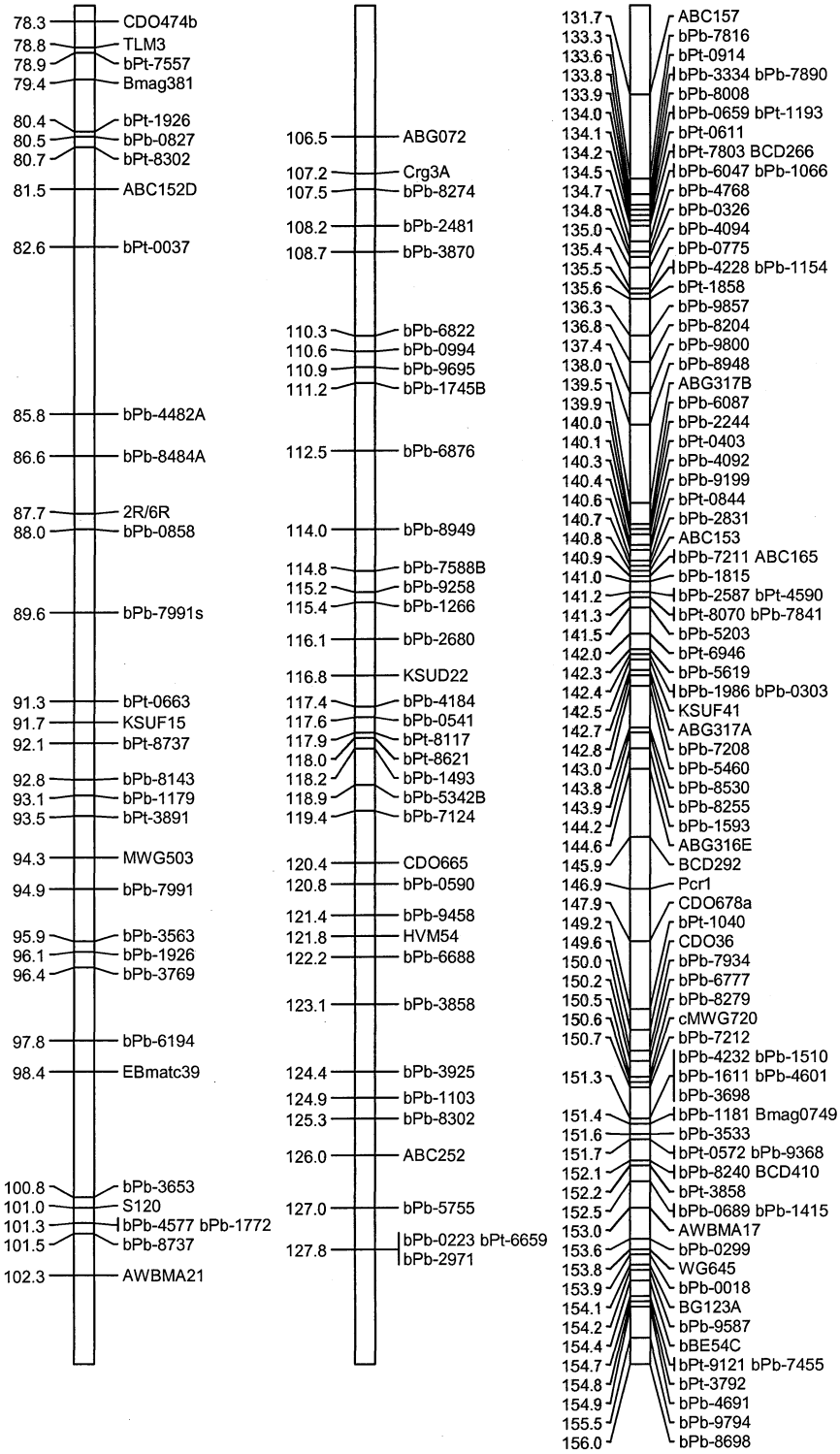
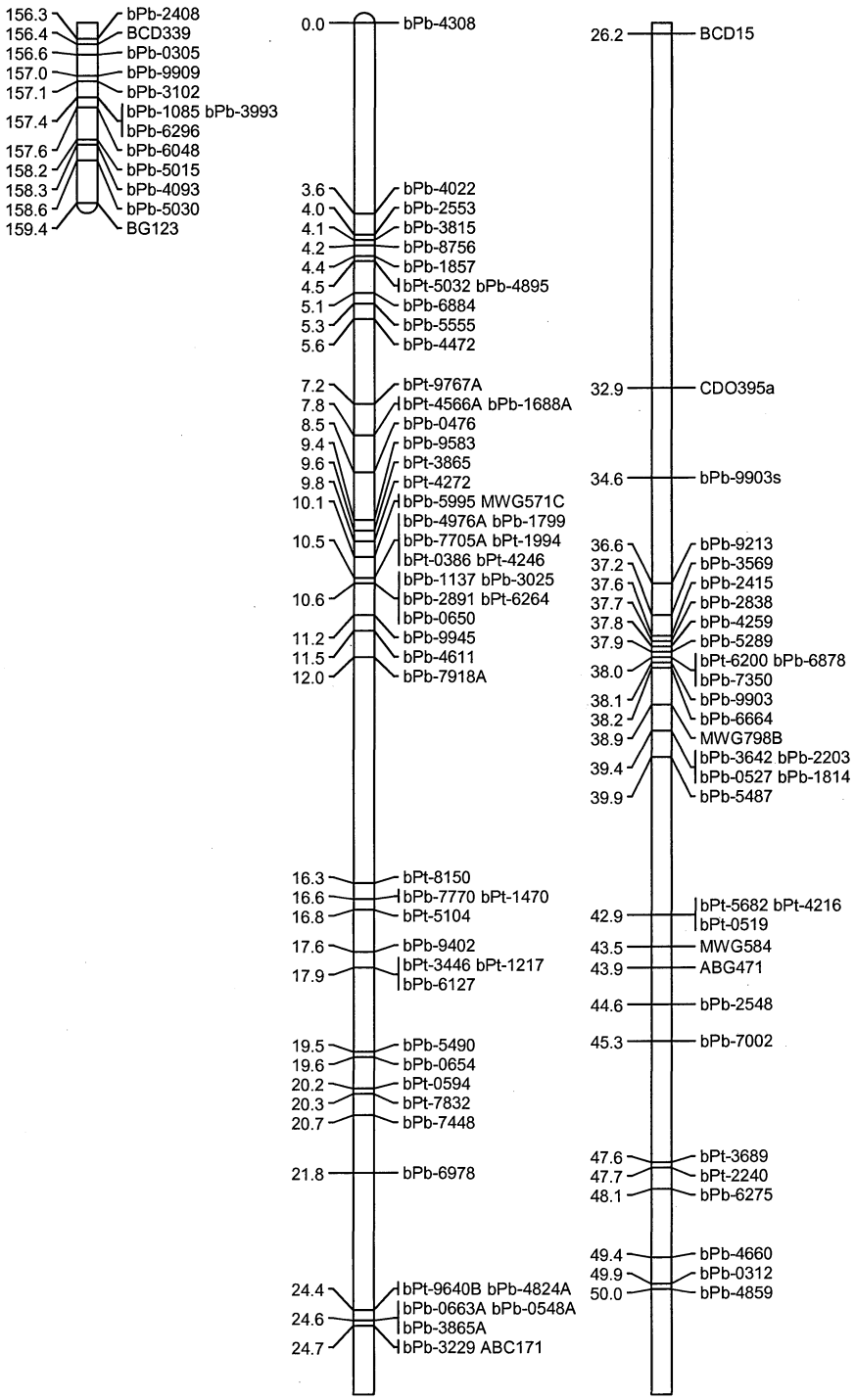
137.4 bPb-7137
137.7 bPb-7697
138.0 bPb-0405
138.5 bPt-3470
138.6 bPb-1312
138.7 bPb-3776
138.9 bPb-1562
139.1 bPt-8272
140.1 bPb-9881 bPb-3622
140.2 bPb-1348
140.3 bPt-1398
140.6 bPb-0181
141.0 bPb-6238
141.5 bPb-5064 bPb-6451
145.6 bPb-0878s bPb-0878c
148.4 bPb-7973B
150.0 bPb-4741

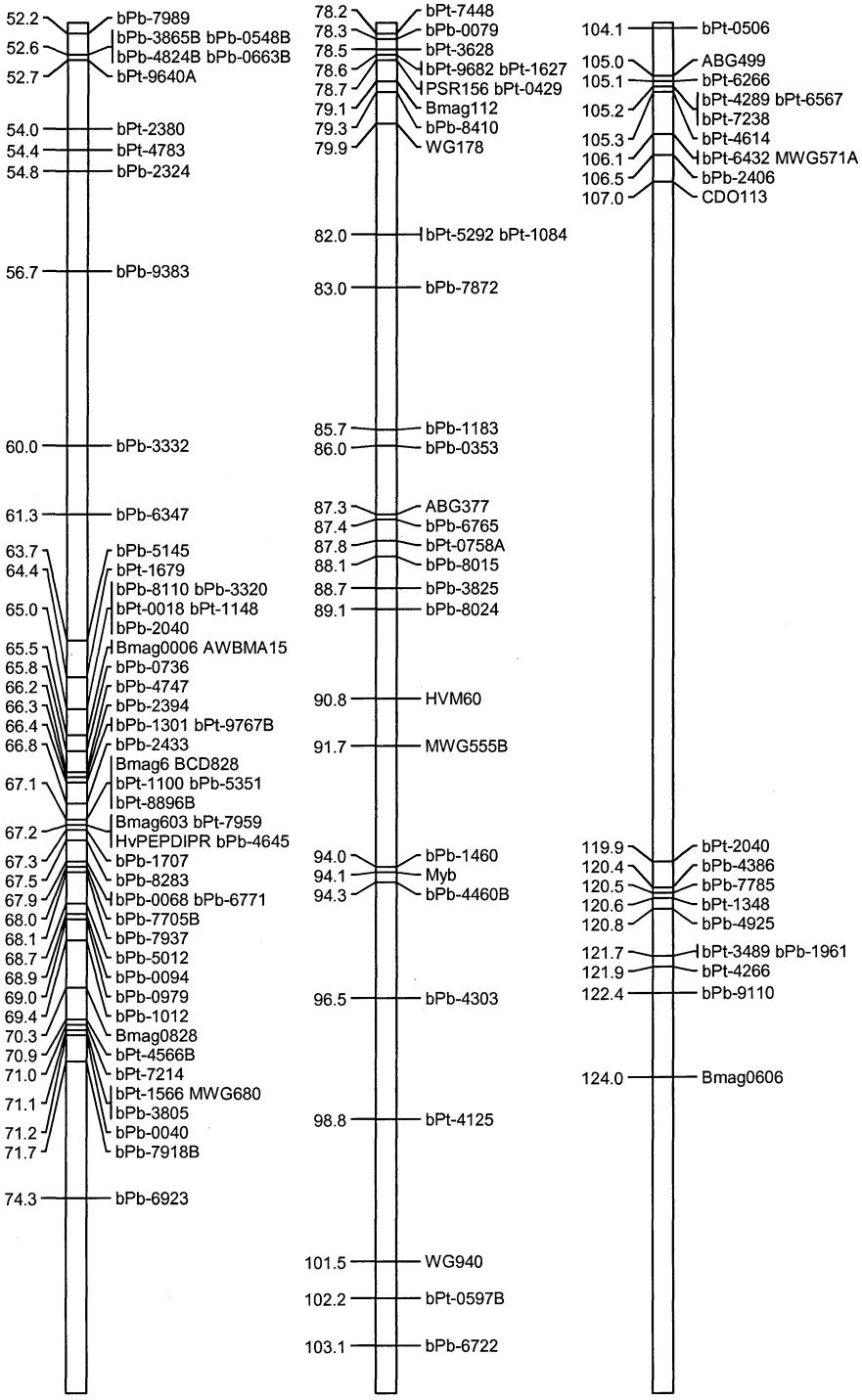
Chromosome 2H



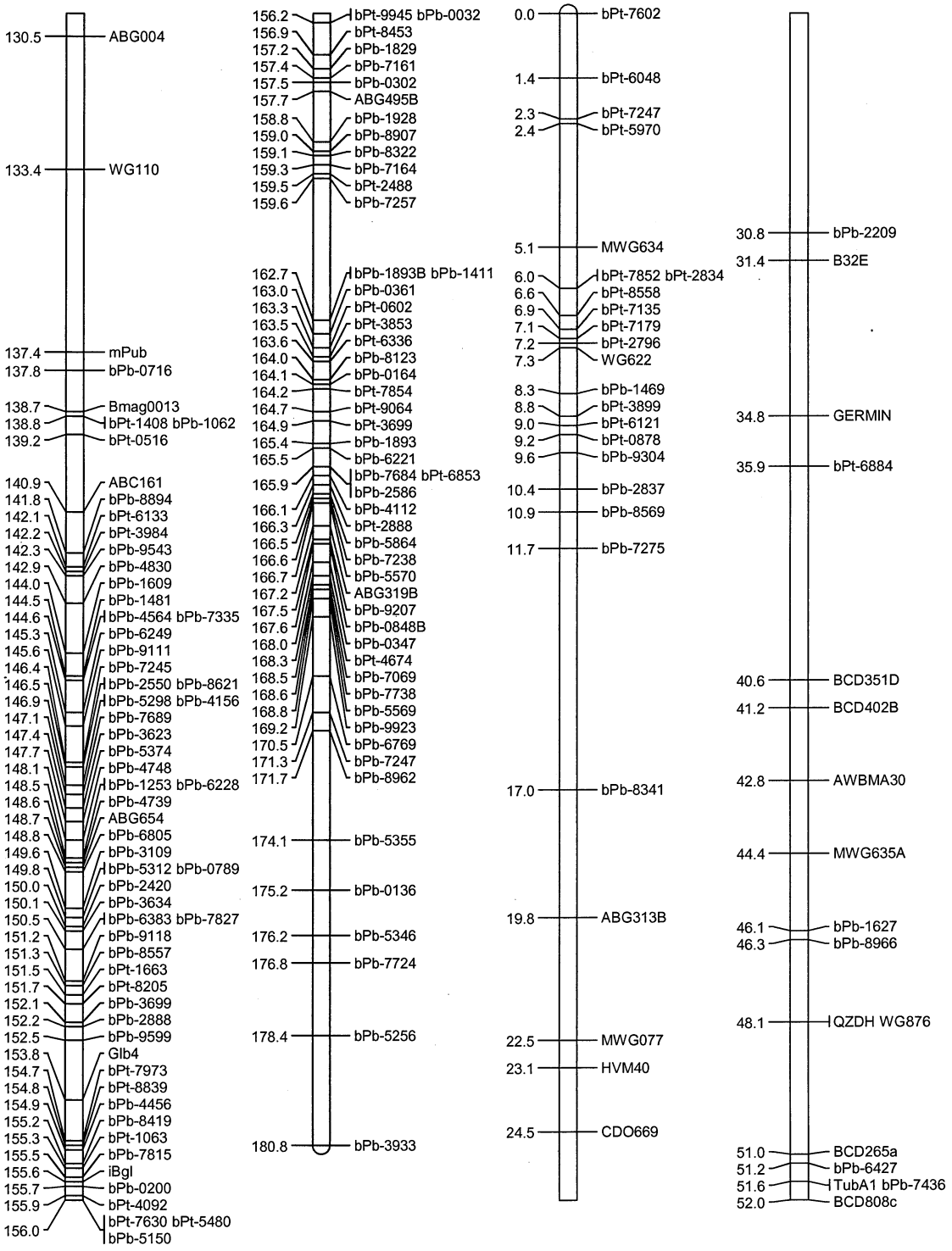

78.3 CDO474b
78.8 TLM3
78.9 bPt-7557
79.4 Bmag381
80.4 bPt-1926
80.5 bPb-0827
80.7 bPt-8302
81.5 ABC152D
82.6 bPt-0037
85.8 bPb-4482A
86.6 bPb-8484A
87.7 2R/6R
88.0 bPb-0858
89.6 bPb-7991s
91.3 bPt-0663
91.7 KSUF15
92.1 bPt-8737
92.8 bPb-8143
93.1 bPb-1179
93.5 bPt-3891
94.3 MWG503
94.9 bPb-7991
95.9 bPb-3563
96.1 bPb-1926
96.4 bPb-3769
97.8 bPb-6194
98.4 EBmatc39
100.8 bPb-3653
101.0 S120
101.3 bPb-4577 bPb-1772
101.5 bPb-8737
102.3 AWBMA21
106.5 ABG072
107.2 Crg3A
107.5 bPb-8274
108.2 bPb-2481
108.7 bPb-3870
110.3 bPb-6822
110.6 bPb-0994
110.9 bPb-9695
111.2 bPb-1745B
112.5 bPb-6876
114.0 bPb-8949
114.8 bPb-7588B
115.2 bPb-9258
115.4 bPb-1266
116.1 bPb-2680
116.8 KSUD22
117.4 bPb-4184
117.6 bPb-0541
117.9 bPt-8117
118.0 bPt-8621
118.2 bPb-1493
118.9 bPb-5342B
119.4 bPb-7124
120.4 CDO665
120.8 bPb-0590
121.4 bPb-9458
121.8 HVM54
122.2 bPb-6688
123.1 bPb-3858
124.4 bPb-3925
124.9 bPb-1103
125.3 bPb-8302
126.0 ABC252
127.0 bPb-5755
127.8 bPb-0223 bPt-6659
bPb-2971
131.7 ABC157
133.3 bPb-7816
133.6 bPt-0914
133.8 bPb-3334 bPb-7890
133.9 bPb-8008
134.0 bPb-0659 bPt-1193
134.1 bPt-0611
134.2 bPt-7803 BCD266
134.5 bPb-6047 bPb-1066
134.7 bPb-4768
134.8 bPb-0326
135.0 bPb-4094
135.4 bPb-0775
135.5 bPb-4228 bPb-1154
135.6 bPt-1858
136.3 bPb-9857
136.8 bPb-8204
137.4 bPb-9800
138.0 bPb-8948
139.5 ABG317B
139.9 bPb-6087
140.0 bPb-2244
140.1 bPt-0403
140.3 bPb-4092
140.4 bPb-9199
140.6 bPt-0844
140.7 bPb-2831
140.8 ABC153
140.9 bPb-7211 ABC165
141.0 bPb-1815
141.2 bPb-2587 bPt-4590
141.3 bPt-8070 bPb-7841
141.5 bPb-5203
142.0 bPt-6946
142.3 bPb-5619
142.4 bPb-1986 bPb-0303
142.5 KSUF41
142.7 ABG317A
142.8 bPb-7208
143.0 bPb-5460
143.8 bPb-8530
143.9 bPb-8255
144.2 bPb-1593
144.6 ABG316E
145.9 BCD292
146.9 Pcr1
147.9 CDO678a
149.2 bPt-1040
149.6 CDO36
150.0 bPb-7934
150.2 bPb-6777
150.5 bPb-8279
150.6 cMWG720
150.7 bPb-7212
bPb-4232 bPb-1510
151.3 bPb-1611 bPb-4601
bPb-3698
151.4 bPb-1181 Bmag0749
151.6 bPb-3533
151.7 bPt-0572 bPb-9368
152.1 bPb-8240 BCD410
152.2 bPt-3858
152.5 bPb-0689 bPb-1415
153.0 AWBMA17
153.6 bPb-0299
153.8 WG645
153.9 bPb-0018
154.1 BG123A
154.2 bPb-9587
154.4 bBE54C
154.7 bPt-9121 bPb-7455
154.8 bPt-3792
154.9 bPb-4691
155.5 bPb-9794
156.0 bPb-8698

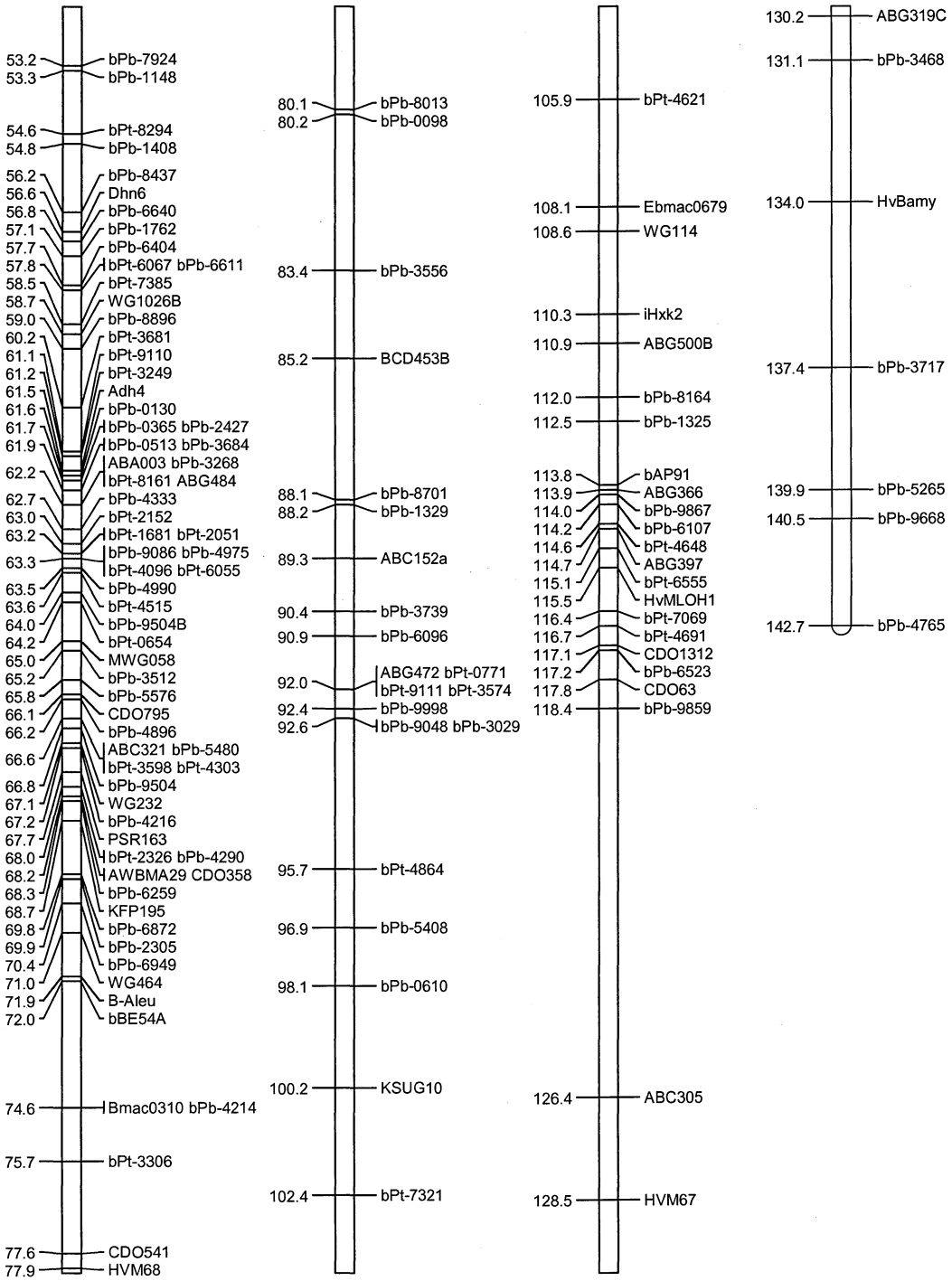
Chromosome 3H



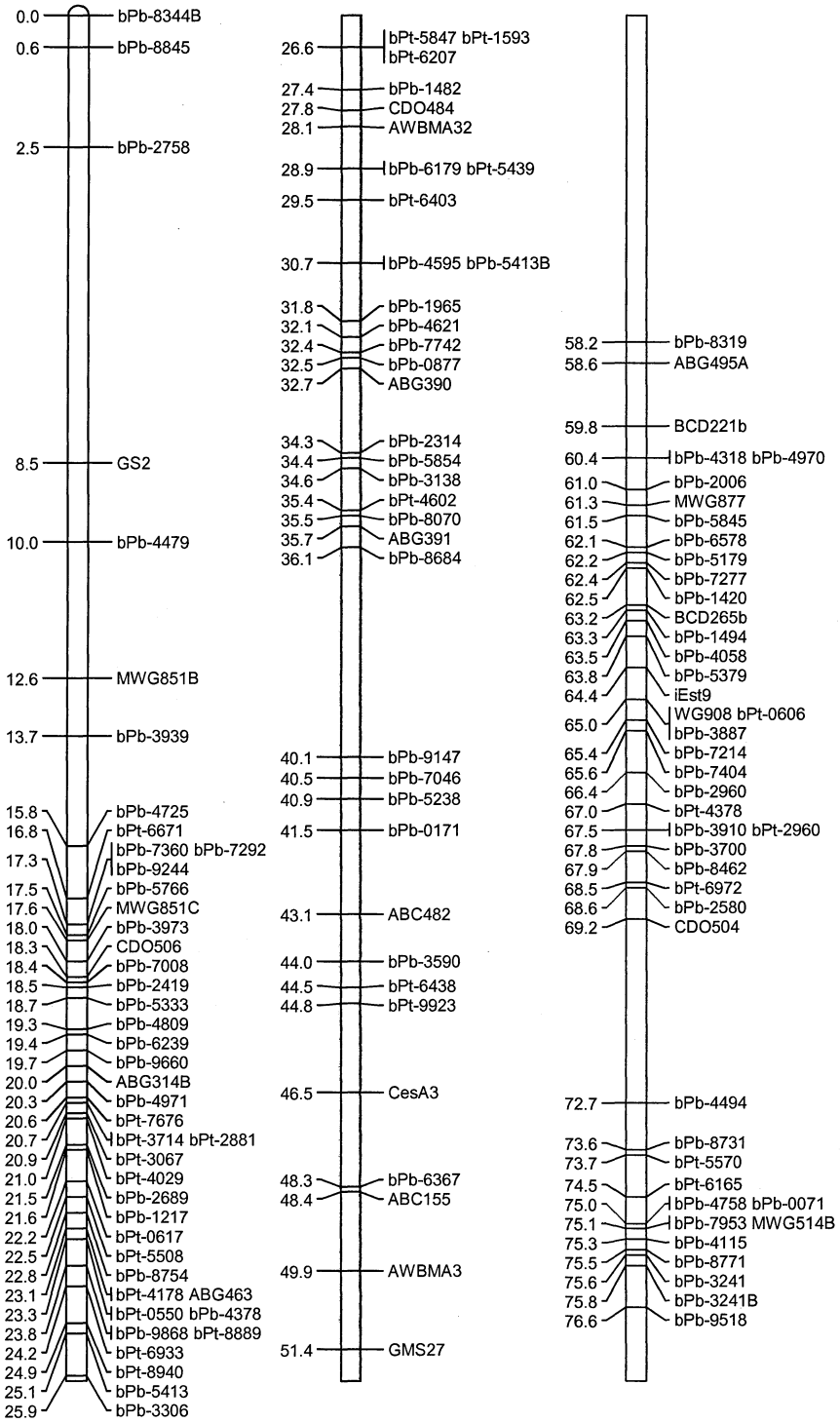

52.2 bPb-7989
52.6 bPb-3865B bPb-0548B
bPb-4824B bPb-0663B
52.7 bPt-9640A
54.0 bPt-2380
54.4 bPt-4783
54.8 bPb-2324
56.7 bPb-9383
60.0 bPb-3332
61.3 bPb-6347
63.7 bPb-5145
64.4 bPt-1679
bPb-8110 bPb-3320
65.0 bPt-0018 bPt-1148
bPb-2040
65.5 Bmag0006 AWBMA15
65.8 bPb-0736
66.2 bPb-4747
66.3 bPb-2394
66.4 bPb-1301 bPt-9767B
66.8 bPb-2433
Bmag6 BCD828
67.1 bPt-1100 bPb-5351
bPt-8896B
67.2 Bmag603 bPt-7959
HvPEPDIPR bPb-4645
67.3 bPb-1707
67.5 bPb-8283
67.9 bPb-0068 bPb-6771
68.0 bPb-7705B
68.1 bPb-7937
68.7 bPb-5012
68.9 bPb-0094
69.0 bPb-0979
69.4 bPb-1012
70.3 Bmag0828
70.9 bPt-4566B
71.0 bPb-7214
71.1 bPt-1566 MWG680
bPb-3805
71.2 bPb-0040
71.7 bPb-7918B
74.3 bPb-6923
78.2 bPt-7448
78.3 bPb-0079
78.5 bPt-3628
78.6 bPt-9682 bPt-1627
78.7 PSR156 bPt-0429
79.1 Bmag112
79.3 bPb-8410
79.9 WG178
82.0 bPt-5292 bPt-1084
83.0 bPb-7872
85.7 bPb-1183
86.0 bPb-0353
87.3 ABG377
87.4 bPb-6765
87.8 bPt-0758A
88.1 bPb-8015
88.7 bPb-3825
89.1 bPb-8024
90.8 HVM60
91.7 MWG555B
94.0 bPb-1460
94.1 Myb
94.3 bPb-4460B
96.5 bPb-4303
98.8 bPt-4125
101.5 WG940
102.2 bPt-0597B
103.1 bPb-6722
104.1 bPt-0506
105.0 ABG499
105.1 bPt-6266
bPt-4289 bPt-6567
105.2 bPt-7238
105.3 bPt-4614
106.1 bPt-6432 MWG571A
106.5 bPb-2406
107.0 CDO113
119.9 bPt-2040
120.4 bPb-4386
120.5 bPb-7785
120.6 bPt-1348
120.8 bPb-4925
121.7 bPt-3489 bPb-1961
121.9 bPt-4266
122.4 bPb-9110
124.0 Bmag0606

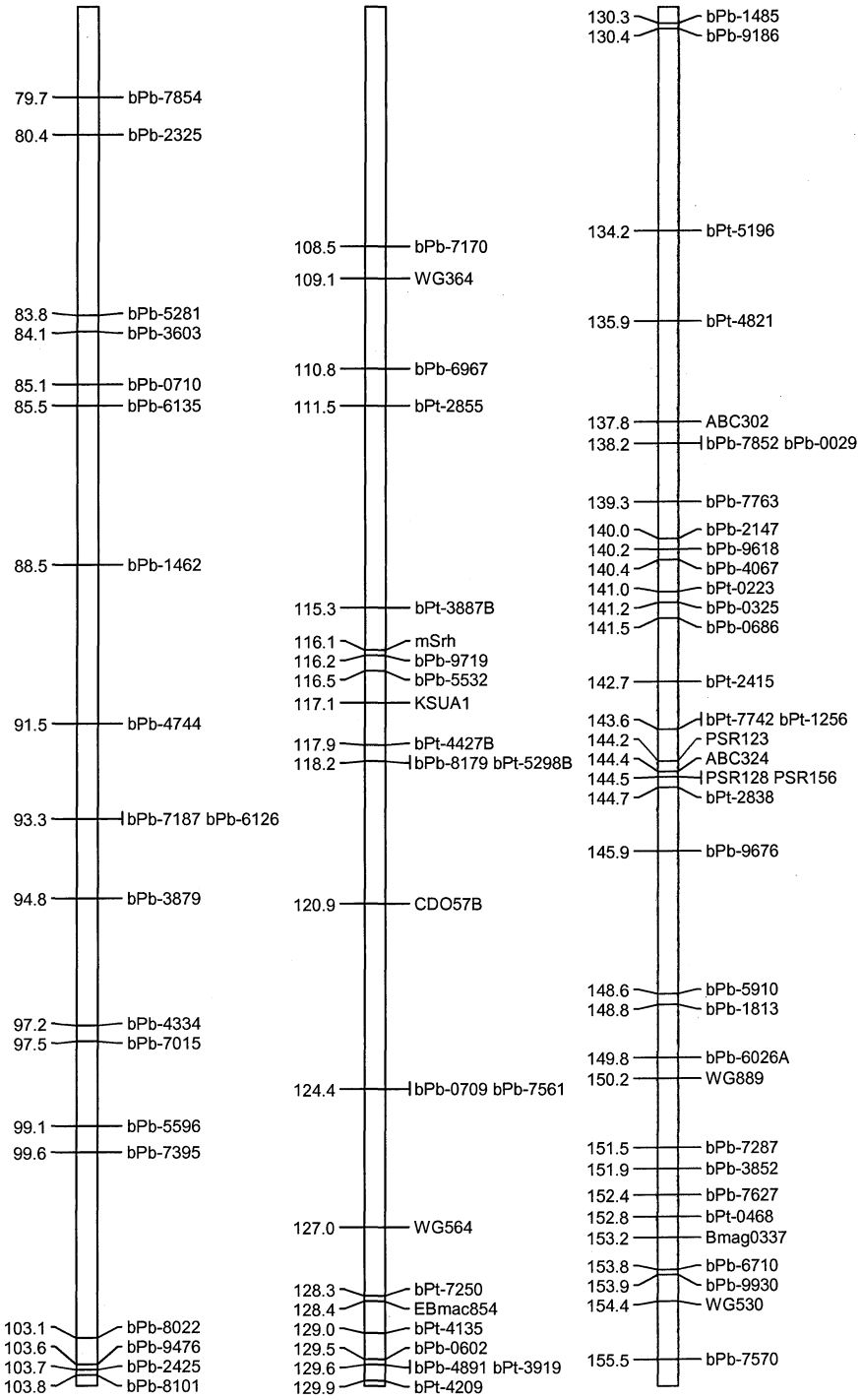
Chromosome 4H

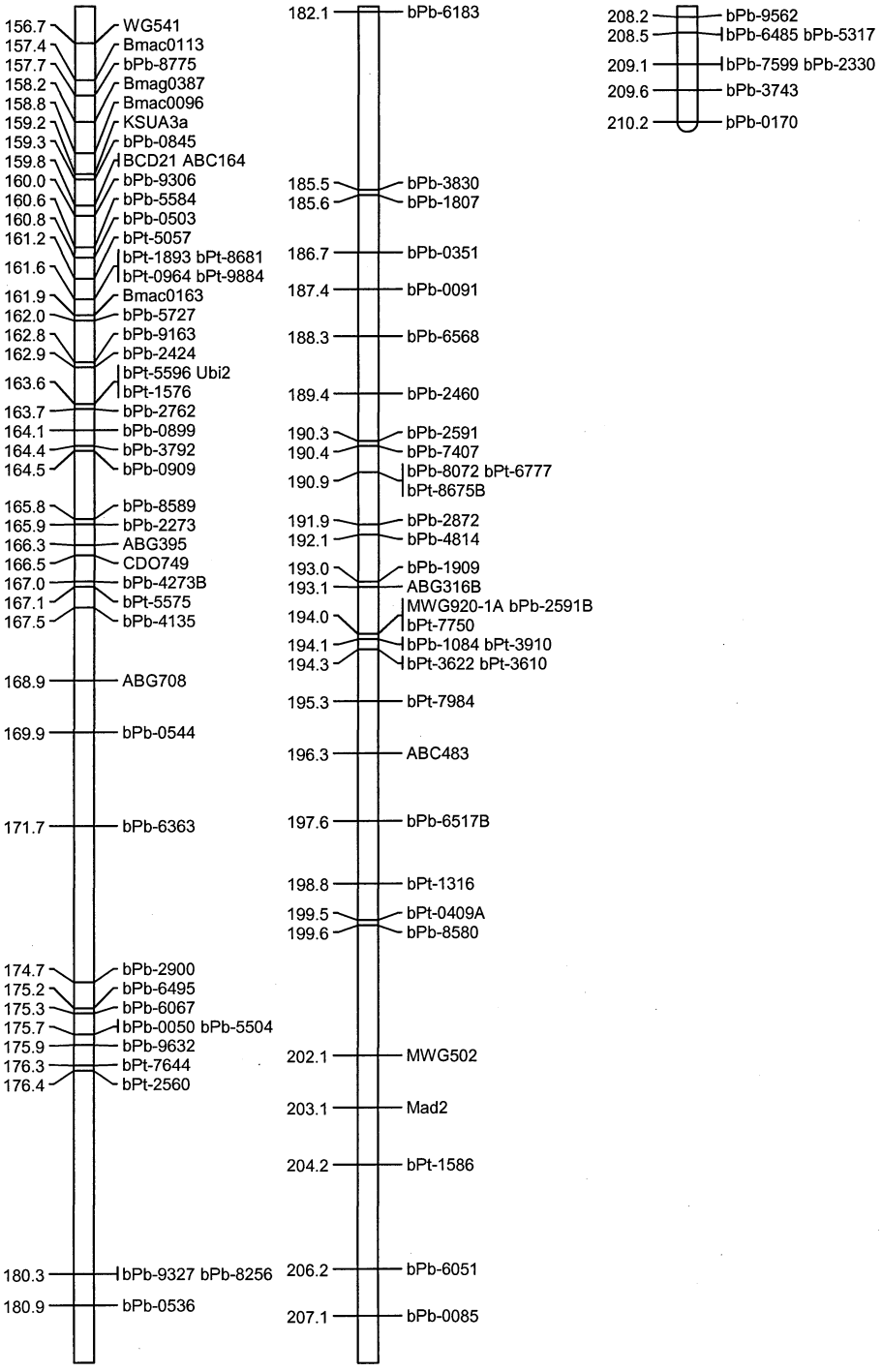



53.2 bPb-7924
53.3 bPb-1148
54.6 bPt-8294
54.8 bPb-1408
56.2 bPb-8437
56.6 Dhn6
56.8 bPb-6640
57.1 bPb-1762
57.7 bPb-6404
57.8 bPt-6067 bPb-6611
58.5 bPt-7385
58.7 WG1026B
59.0 bPb-8896
60.2 bPt-3681
61.1 bPt-9110
61.2 bPt-3249
61.5 Adh4
61.6 bPb-0130
61.7 bPb-0365 bPb-2427
61.9 bPb-0513 bPb-3684
62.2 ABA003 bPb-3268
bPt-8161 ABG484
62.7 bPb-4333
63.0 bPt-2152
63.2 bPt-1681 bPt-2051
63.3 bPb-9086 bPb-4975
bPt-4096 bPt-6055
63.5 bPb-4990
63.6 bPt-4515
64.0 bPb-9504B
64.2 bPt-0654
65.0 MWG058
65.2 bPb-3512
65.8 bPb-5576
66.1 CDO795
66.2 bPb-4896
66.6 ABC321 bPb-5480
bPt-3598 bPt-4303
66.8 bPb-9504
67.1 WG232
67.2 bPb-4216
67.7 PSR163
68.0 bPt-2326 bPb-4290
68.2 AWBMA29 CDO358
68.3 bPb-6259
68.7 KFP195
69.8 bPb-6872
69.9 bPb-2305
70.4 bPb-6949
71.0 WG464
71.9 B-Aleu
72.0 bBE54A
74.6 Bmac0310 bPb-4214
75.7 bPt-3306
77.6 CDO541
77.9 HVM68
80.1 bPb-8013
80.2 bPb-0098
83.4 bPb-3556
85.2 BCD453B
88.1 bPb-8701
88.2 bPb-1329
89.3 ABC152a
90.4 bPb-3739
90.9 bPb-6096
92.0 ABG472 bPt-0771
bPt-9111 bPt-3574
92.4 bPb-9998
92.6 bPb-9048 bPb-3029
95.7 bPt-4864
96.9 bPb-5408
98.1 bPb-0610
100.2 KSUG10
102.4 bPt-7321
105.9 bPt-4621
108.1 Ebmac0679
108.6 WG114
110.3 iHxk2
110.9 ABG500B
112.0 bPb-8164
112.5 bPb-1325
113.8 bAP91
113.9 ABG366
114.0 bPb-9867
114.2 bPb-6107
114.6 bPt-4648
114.7 ABG397
115.1 bPt-6555
115.5 HvMLOH1
116.4 bPt-7069
116.7 bPt-4691
117.1 CDO1312
117.2 bPb-6523
117.8 CDO63
118.4 bPb-9859
126.4 ABC305
128.5 HVM67
130.2 ABG319C
131.1 bPb-3468
134.0 HvBamy
137.4 bPb-3717
139.9 bPb-5265
140.5 bPb-9668
142.7 bPb-4765

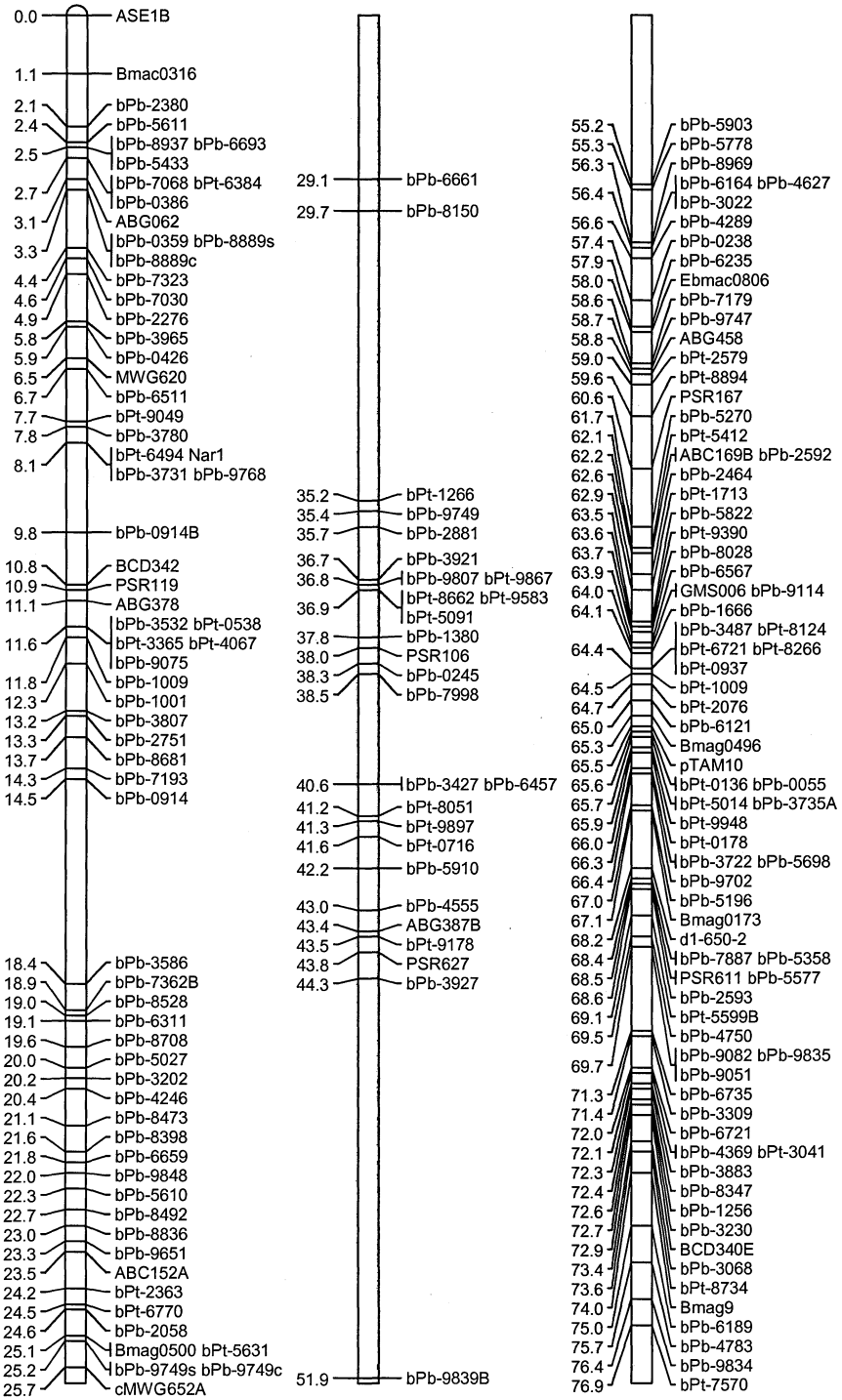
Chromosome 5H

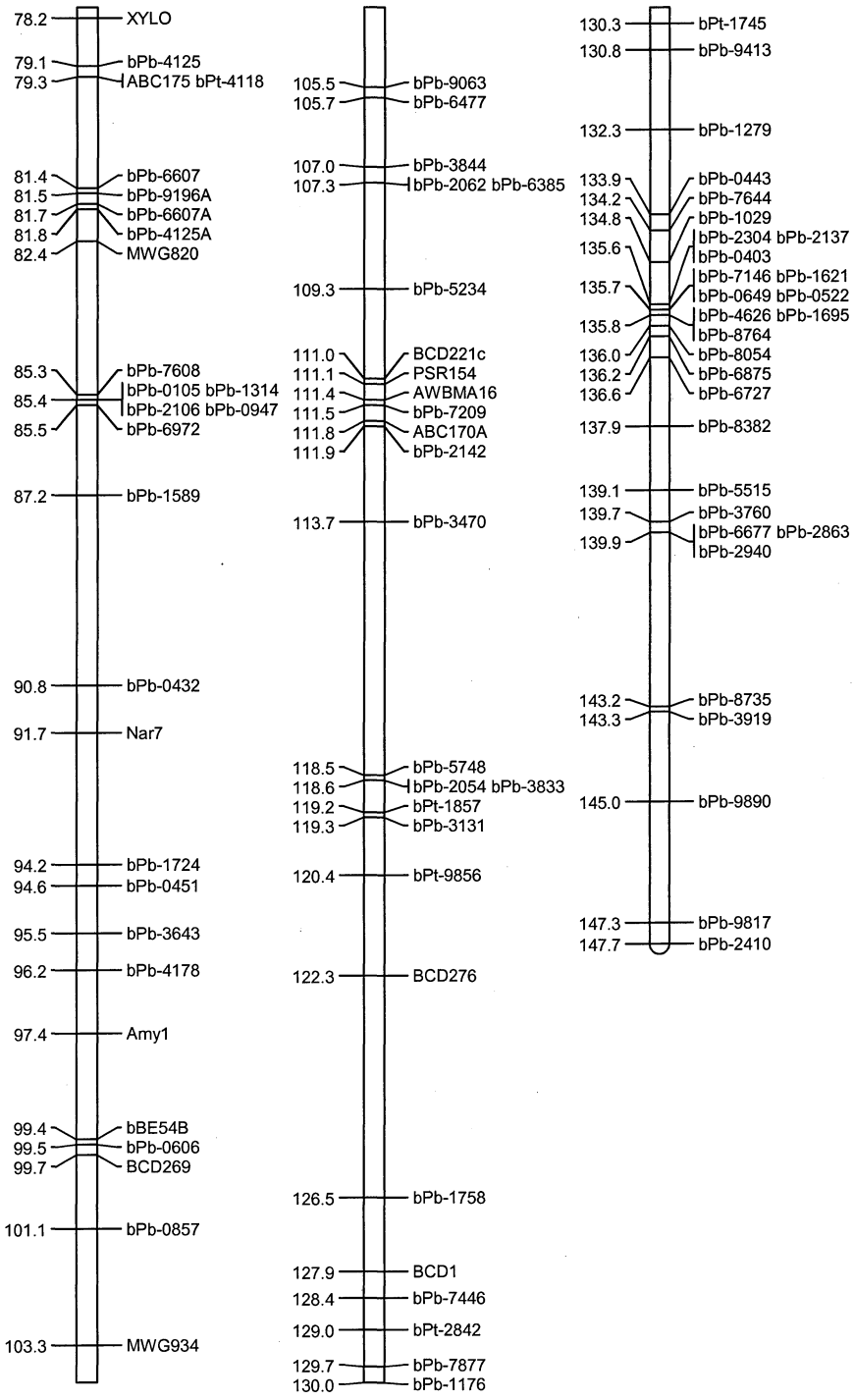



79.7 bPb-7854
80.4 bPb-2325
83.8 bPb-5281
84.1 bPb-3603
85.1 bPb-0710
85.5 bPb-6135
88.5 bPb-1462
91.5 bPb-4744
93.3 bPb-7187 bPb-6126
94.8 bPb-3879
97.2 bPb-4334
97.5 bPb-7015
99.1 bPb-5596
99.6 bPb-7395
103.1 bPb-8022
103.6 bPb-9476
103.7 bPb-2425
103.8 bPb-8101
108.5 bPb-7170
109.1 WG364
110.8 bPb-6967
111.5 bPt-2855
115.3 bPt-3887B
116.1 mSrh
116.2 bPb-9719
116.5 bPb-5532
117.1 KSUA1
117.9 bPt-4427B
118.2 bPb-8179 bPt-5298B
120.9 CDO57B
124.4 bPb-0709 bPb-7561
127.0 WG564
128.3 bPt-7250
128.4 EBmac854
129.0 bPt-4135
129.5 bPb-0602
129.6 bPb-4891 bPt-3919
129.9 bPt-4209
130.3 bPb-1485
130.4 bPb-9186
134.2 bPt-5196
135.9 bPt-4821
137.8 ABC302
138.2 bPb-7852 bPb-0029
139.3 bPb-7763
140.0 bPb-2147
140.2 bPb-9618
140.4 bPb-4067
141.0 bPt-0223
141.2 bPb-0325
141.5 bPb-0686
142.7 bPt-2415
143.6 bPt-7742 bPt-1256
144.2 PSR123
144.4 ABC324
144.5 PSR128 PSR156
144.7 bPt-2838
145.9 bPb-9676
148.6 bPb-5910
148.8 bPb-1813
149.8 bPb-6026A
150.2 WG889
151.5 bPb-7287
151.9 bPb-3852
152.4 bPb-7627
152.8 bPt-0468
153.2 Bmag0337
153.8 bPb-6710
153.9 bPb-9930
154.4 WG530
155.5 bPb-7570

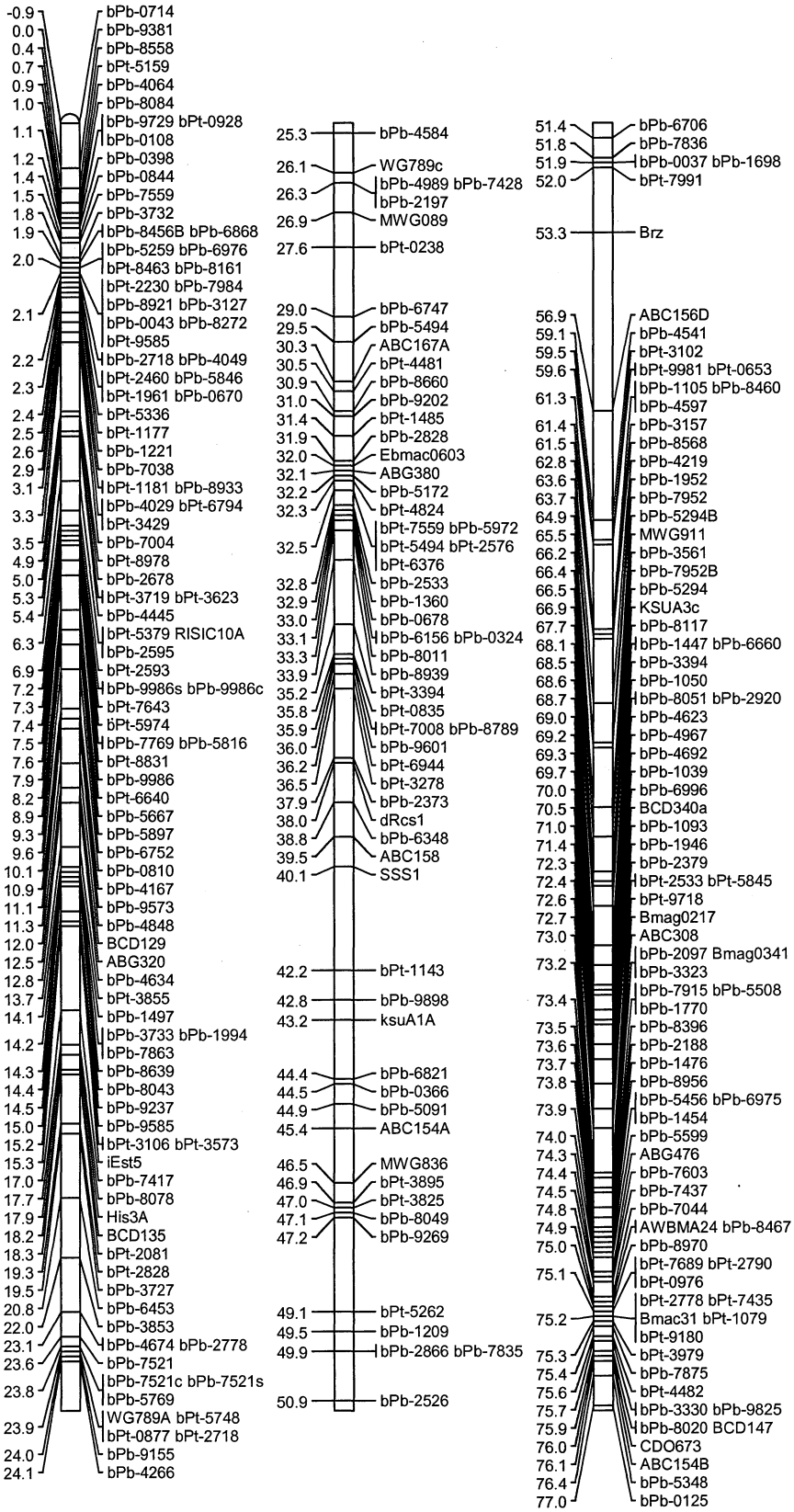

156.7 WG541
157.4 Bmac0113
157.7 bPb-8775
158.2 Bmag0387
158.8 Bmac0096
159.2 KSUA3a
159.3 bPb-0845
159.8 BCD21 ABC164
160.0 bPb-9306
160.6 bPb-5584
160.8 bPb-0503
161.2 bPt-5057
161.6 bPt-1893 bPt-8681
bPt-0964 bPt-9884
161.9 Bmac0163
162.0 bPb-5727
162.8 bPb-9163
162.9 bPb-2424
163.6 bPt-5596 Ubi2
bPt-1576
163.7 bPb-2762
164.1 bPb-0899
164.4 bPb-3792
164.5 bPb-0909
165.8 bPb-8589
165.9 bPb-2273
166.3 ABG395
166.5 CDO749
167.0 bPb-4273B
167.1 bPt-5575
167.5 bPb-4135
168.9 ABG708
169.9 bPb-0544
171.7 bPb-6363
174.7 bPb-2900
175.2 bPb-6495
175.3 bPb-6067
175.7 bPb-0050 bPb-5504
175.9 bPb-9632
176.3 bPt-7644
176.4 bPt-2560
180.3 bPb-9327 bPb-8256
180.9 bPb-0536
182.1 bPb-6183
185.5 bPb-3830
185.6 bPb-1807
186.7 bPb-0351
187.4 bPb-0091
188.3 bPb-6568
189.4 bPb-2460
190.3 bPb-2591
190.4 bPb-7407
190.9 bPb-8072 bPt-6777
bPt-8675B
191.9 bPb-2872
192.1 bPb-4814
193.0 bPb-1909
193.1 ABG316B
194.0 MWG920-1A bPb-2591B
bPt-7750
194.1 bPb-1084 bPt-3910
194.3 bPt-3622 bPt-3610
195.3 bPt-7984
196.3 ABC483
197.6 bPb-6517B
198.8 bPt-1316
199.5 bPt-0409A
199.6 bPb-8580
202.1 MWG502
203.1 Mad2
204.2 bPt-1586
206.2 bPb-6051
207.1 bPb-0085
208.2 bPb-9562
208.5 bPb-6485 bPb-5317
209.1 bPb-7599 bPb-2330
209.6 bPb-3743
210.2 bPb-0170

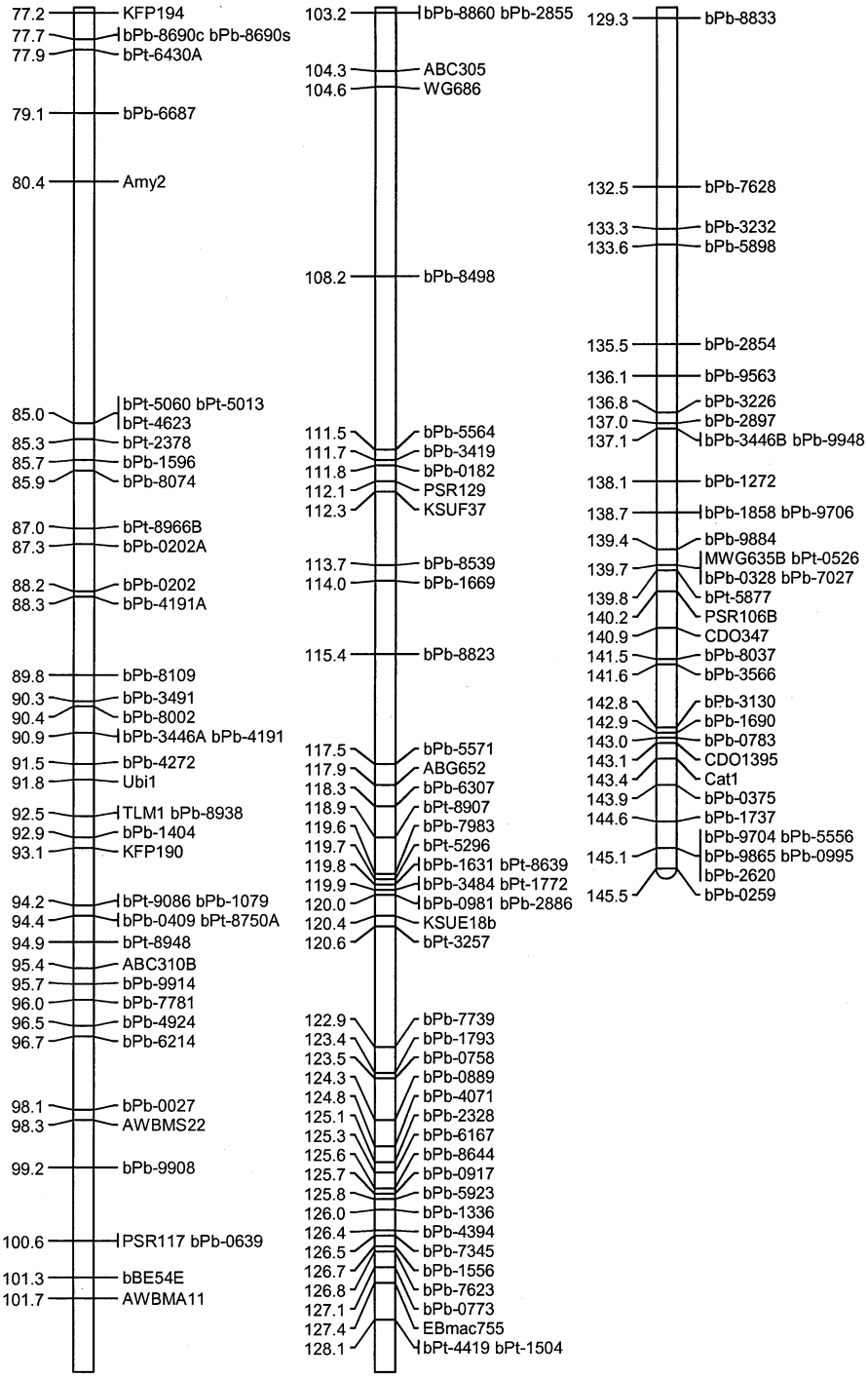
Chromosome 6H




78.2 XYLO
79.1 bPb-4125
79.3 ABC175 bPt-4118
81.4 bPb-6607
81.5 bPb-9196A
81.7 bPb-6607A
81.8 bPb-4125A
82.4 MWG820
85.3 bPb-7608
85.4 bPb-0105 bPb-1314
bPb-2106 bPb-0947
85.5 bPb-6972
87.2 bPb-1589
90.8 bPb-0432
91.7 Nar7
94.2 bPb-1724
94.6 bPb-0451
95.5 bPb-3643
96.2 bPb-4178
97.4 Amy1
99.4 bBE54B
99.5 bPb-0606
99.7 BCD269
101.1 bPb-0857
103.3 MWG934
105.5 bPb-9063
105.7 bPb-6477
107.0 bPb-3844
107.3 bPb-2062 bPb-6385
109.3 bPb-5234
111.0 BCD221c
111.1 PSR154
111.4 AWBMA16
111.5 bPb-7209
111.8 ABC170A
111.9 bPb-2142
113.7 bPb-3470
118.5 bPb-5748
118.6 bPb-2054 bPb-3833
119.2 bPt-1857
119.3 bPb-3131
120.4 bPt-9856
122.3 BCD276
126.5 bPb-1758
127.9 BCD1
128.4 bPb-7446
129.0 bPt-2842
129.7 bPb-7877
130.0 bPb-1176
130.3 bPt-1745
130.8 bPb-9413
132.3 bPb-1279
133.9 bPb-0443
134.2 bPb-7644
134.8 bPb-1029
135.6 bPb-2304 bPb-2137
bPb-0403
135.7 bPb-7146 bPb-1621
bPb-0649 bPb-0522
135.8 bPb-4626 bPb-1695
bPb-8764
136.0 bPb-8054
136.2 bPb-6875
136.6 bPb-6727
137.9 bPb-8382
139.1 bPb-5515
139.7 bPb-3760
139.9 bPb-6677 bPb-2863
bPb-2940
143.2 bPb-8735
143.3 bPb-3919
145.0 bPb-9890
147.3 bPb-9817
147.7 bPb-2410

Chromosome 7H




77.2 KFP194
77.7 bPb-8690c bPb-8690s
77.9 bPt-6430A
79.1 bPb-6687
80.4 Amy2
85.0 bPt-5060 bPt-5013
bPt-4623
85.3 bPt-2378
85.7 bPb-1596
85.9 bPb-8074
87.0 bPt-8966B
87.3 bPb-0202A
88.2 bPb-0202
88.3 bPb-4191A
89.8 bPb-8109
90.3 bPb-3491
90.4 bPb-8002
90.9 bPb-3446A bPb-4191
91.5 bPb-4272
91.8 Ubi1
92.5 TLM1 bPb-8938
92.9 bPb-1404
93.1 KFP190
94.2 bPt-9086 bPb-1079
94.4 bPb-0409 bPt-8750A
94.9 bPt-8948
95.4 ABC310B
95.7 bPb-9914
96.0 bPb-7781
96.5 bPb-4924
96.7 bPb-6214
98.1 bPb-0027
98.3 AWBMS22
99.2 bPb-9908
100.6 PSR117 bPb-0639
101.3 bBE54E
101.7 AWBMA11
103.2 bPb-8860 bPb-2855
104.3 ABC305
104.6 WG686
108.2 bPb-8498
111.5 bPb-5564
111.7 bPb-3419
111.8 bPb-0182
112.1 PSR129
112.3 KSUF37
113.7 bPb-8539
114.0 bPb-1669
115.4 bPb-8823
117.5 bPb-5571
117.9 ABG652
118.3 bPb-6307
118.9 bPt-8907
119.6 bPb-7983
119.7 bPt-5296
119.8 bPb-1631 bPt-8639
119.9 bPb-3484 bPt-1772
120.0 bPb-0981 bPb-2886
120.4 KSUE18b
120.6 bPt-3257
122.9 bPb-7739
123.4 bPb-1793
123.5 bPb-0758
124.3 bPb-0889
124.8 bPb-4071
125.1 bPb-2328
125.3 bPb-6167
125.6 bPb-8644
125.7 bPb-0917
125.8 bPb-5923
126.0 bPb-1336
126.4 bPb-4394
126.5 bPb-7345
126.7 bPb-1556
126.8 bPb-7623
127.1 bPb-0773
127.4 EBmac755
128.1 bPt-4419 bPt-1504
129.3 bPb-8833
132.5 bPb-7628
133.3 bPb-3232
133.6 bPb-5898
135.5 bPb-2854
136.1 bPb-9563
136.8 bPb-3226
137.0 bPb-2897
137.1 bPb-3446B bPb-9948
138.1 bPb-1272
138.7 bPb-1858 bPb-9706
139.4 bPb-9884
139.7 MWG635B bPt-0526
bPb-0328 bPb-7027
139.8 bPt-5877
140.2 PSR106B
140.9 CDO347
141.5 bPb-8037
141.6 bPb-3566
142.8 bPb-3130
142.9 bPb-1690
143.0 bPb-0783
143.1 CDO1395
143.4 Cat1
143.9 bPb-0375
144.6 bPb-1737
145.1 bPb-9704 bPb-5556
bPb-9865 bPb-0995
bPb-2620
145.5 bPb-0259